2003

# Evaluation of prairie grasses for reducing the environmental impact of herbicide contamination

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# Evaluation of prairie grasses for reducing the environmental impact of herbicide contamination

by

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A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Toxicology

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Iowa State University
Ames, Iowa
2003

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# ABSTRACT

The primary goal of this dissertation was to evaluate the use of prairie grasses for reducing the environmental impact of herbicides. Studies included: use of prairie grasses as a phytoremediation tool for contaminated soil; comparison of grass species for use in vegetative buffer strips; fate of $^{14}C$-pendimethalin in unvegetated and unvegetated soil; and environmental hazards of pendimethalin contaminated soil.

Throughout this dissertation, evidence was presented that prairie grasses can increase the dissipation rate of herbicides. In one study, 78% less metolachlor and 39% less pendimethalin remaining in vegetated treatments as compared to unvegetated treatments. In a separate study, the presence of nearly all grasses tested, but specifically the prairie grasses, resulted in greater degradation of atrazine and metolachlor in rhizosphere soil as compared to unvegetated soil. Phytoremediation mechanisms likely involve plant uptake and increased soil degradation.

Prairie grasses were also shown to decrease movement of pesticides both through the soil column and into biota, thus serving as a phytostabilization agent. Nearly 20% of the metolachlor in unvegetated columns leached out of the bottom of the column after application of an artificial "rain event", while only 5% leached out of vegetated columns. It was also shown that even though vegetated columns allowed infiltration of artificial surface runoff at a much faster rate, the total amount of herbicide moving through the column was held constant, and the amount leaching through after initial applications of herbicide was reduced. Additionally, the presence of vegetation decreased the bioavailability of pendimethalin as measured by earthworm uptake and toxicity to lettuce seedlings.

Pendimethalin residues are very persistent and are likely to be present at some level following bioremediation. Therefore, a hazard evaluation was performed to determine tolerable soil concentrations of pendimethalin that could remain without risk to the biota in the environment. Even low levels of pendimethalin, 10mg/kg or less, were shown to have toxic effects on plants and earthworms, and concentrations as low as 30 mg/kg were shown to have potentially toxic effects through trophic transfer. Thus remediation would need to continue until pendimethalin is reduced to field application levels (10 mg/kg) or less.

# CHAPTER 1. GENERAL INTRODUCTION

Intensive use of pesticides in Iowa has resulted in contaminated soils and water systems. Initial reports from the National Water Quality Assessment conducted by the United States Geological Survey indicate that 95 percent of stream and 50 percent of ground water samples contained detectable levels of at least one pesticide [1]. Most of the pesticides contaminating water systems were intentionally or incidentally applied to soil and then entered surface water as runoff or leached into groundwater. Contamination of groundwater and surface water may pose a threat to aquatic environments and human health. Pesticide residues that remain in soil can also result in environmental risk. Pesticides may accumulate in soil organisms resulting in distribution of the toxicant into the terrestrial food chain, or the pesticide could simply impair the environment by reducing the productivity of the immediate area of the contaminated soil.

Although standard agricultural pesticide use - which may result in non-point source contamination - often is regarded as the primary source of pesticide contamination, point source contamination originating from agrochemical dealerships, manufacturing and formulation facilities, or spills has also become a serious concern [2, 3]. Current technologies for cleanup of pesticide-contaminated soil often involve excavation, which is disruptive to the environment and very expensive. Often soil concentrations at these sites are well above those found at agrochemical dealerships. Many pesticides degrade at slower rates as concentration in soil increases [4, 5], increasing the potential impact of pesticide spills.

The severity of the risk from contaminated soil depends on the concentration, degradation rate, and mobility of the pesticide, in addition to its toxicity. Together these factors describe the potential for the compound to enter biota at a dose high enough to cause an effect. Both degradation rate and mobility of a pesticide in soil are dependent on many factors including the chemical properties of the individual pesticide, chemical and physical properties of the soil, and types and number of biota presence. For example, soils with a high number of pesticide-degrading microbes will have increased degradation rates (increased biodegradation)[6] or a change in soil organic matter quantity or type could change the degree of soil adsorption [7] to a point that the pesticide will less readily leach or move into biota (increased stabilization). By adjusting the soil or types of biota at contaminated sites,

reduction of pesticide transfer to critical environmental compartments (e.g. biota or groundwater) may be possible.

The types and amount of vegetation present can have a significant impact on both soil properties and biota present, potentially influencing the degradation patterns of organic contaminants. Plant material incorporated into the soil or released from roots can affect the cycling of organic matter and other nutrients [8]. Pores around roots and uptake and transpiration of water can affect the movement of water in the soil column. Exudates released in the root zone (rhizosphere) contain a variety of carbon and sometimes nitrogen compounds that greatly increase the potential for microbial activity in the rhizosphere [8], which may increase pesticide degradation, often cometabolically [6]. In addition, combinations of these processes can help increase soil structure. Each of these changes could influence the amount of contaminant available to leaching or for uptake by biota. Cycling of organic matter and more complex soil structure may increase the adsorption of a contaminant onto surfaces that are unavailable for degradation by microorganisms or leaching by moving water, resulting in stabilization of the contaminant (or phytostabilization, as the process was initiated by a plant). Plant uptake can also increase the removal and biotransformation the contaminants [9, 10].

For several years, researchers have advocated the use of vegetative buffer strips for removal of pesticides from runoff [11], and more recently studies have been conducted on the use of plants for remediation of soil contaminated with organic compounds (phytoremediation) [12, 13]. While the use of plants for controlling a contaminant's distribution or degradation is not a new idea, the processes involved are not understood and the technology is far from optimized. In the case of buffer strips, most projects have been large-scale field procedures that cite infiltration as the primary route for reduction of pesticides in the runoff from fields [11, 14]. However, few projects have tried to track the fate of the pesticides in the soil column after infiltration. Even fewer projects have compared several different types of vegetation for their relative ability to degrade pesticides within the soil column. In the case of phytoremediation, many studies have been conducted for the remediation of petroleum products and industrial solvents [13, 15]. In contrast, few studies have investigated the phytoremediation of pesticides, especially herbicides, although several have noted enhanced pesticide degradation in soil collected from the root zone

(rhizosphere)[12,16]. In addition only a few plants have been investigated fully for their phytoremediation potential [13]. In research on both vegetative buffer strips and phytoremediation, the total fate of the pesticide is typically not measured, only the dissipation of the parent is determined, even though the formation and toxicity profiles of the metabolites produced in the plant and soil are very important in evaluating the performance of the remediation technique.

Utilizing biological, in addition to chemical, endpoints for measuring the success of remediation projects can provide additional information regarding changes in the contaminated soil's toxicity potential. For instance, if toxicity tests and bioaccumulation studies indicate a decrease in uptake and subsequently toxicity, the bioavailability of the chemical may be decreasing in the soil, therefore decreasing the risks from the contamination [17]. However, if bioassays indicate increased toxicity or accumulation, an increase in bioavailability or production of a toxic metabolite may be occurring in the soil system. Both situations could impact the assessment of the remediation technique; however, neither situation would be identified if only dissipation of the parent compound is measured.

Bioassays are invaluable when conducting a hazard evaluation of the remediated site. When bioremediation techniques are employed, decreases in contaminant level are often realized, but rarely does the technique reduce all contaminants to undetectable levels. Therefore, in order to declare the site "clean enough," determination of the remediated soil's toxicity potential is necessary. Toxicity data are scarce in the literature for many herbicides, especially for meso and macrofauna, and even less information is available for concentrations above levels expected after normal application. A greater amount of soil toxicity data is needed for most herbicides to conduct hazard assessments of contaminated soil.

Throughout this research project, four main herbicides were considered. Each is heavily used in Iowa agriculture. Atrazine and metolachlor are moderately persistent and may leach into groundwater. The other two herbicides, pendimethalin and trifluralin, are in the dinitroanaline class; they are persistent in soil and water (half-life over 90 days) and adsorb tightly to soil, which prevents leaching. Chemical structures are shown in Figure 1 andchemical characteristics, usage data, and environmental occurrence information are listed in Table 1 for each herbicide. Note that each herbicide has been in use for many years

(registration date 25-40 years ago) and that each compound has been used heavily at least from 1987 to 1999. In addition, each of the herbicides, but especially atrazine and metolachlor, are commonly found in ground and surface water.

**Research Goals**

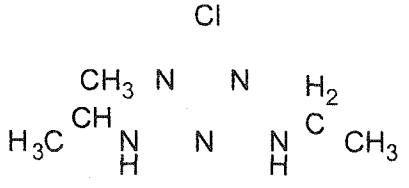
The primary goals of this dissertation were:

1) To further investigate the potential of grasses as mitigating agents for pesticide contaminated soil and surface runoff, specifically to address crucial unanswered questions such as - Does vegetation increase the rate of pesticide dissipation from soil? During the *in situ* remediation process, does contamination leave the soil and disperse into critical environmental matrices such as ground and surface water? Are toxic metabolites produced? Are some grass species better than others?

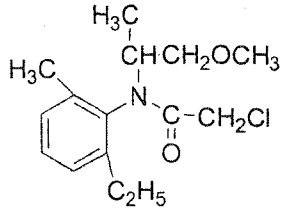
2) To compare biological and chemical endpoints for measuring the success of phytoremediation,

3) To investigate the fate of pendimethalin in vegetated versus unvegetated systems,

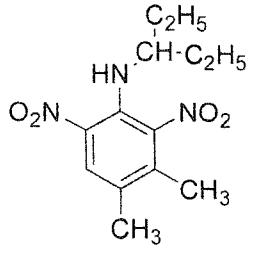
4) To conduct a hazard evaluation of pendimethalin-contaminated soil.



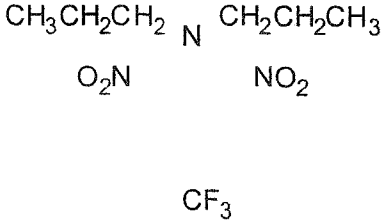
Atrazine



Metolachlor



Pendimethalin



Trifluralin

Figure 1. Chemical structures of primary herbicides discussed.

Table 1. Chemical structure, chemical properties, usage, and occurrence in ground and drinking water for the primary herbicides discussed.

| | **Atrazine** | **Metolachlor** | **Pendimethalin** | **Trifluralin** |
|---|---|---|---|---|
| **Class**[1] | Triazine | Chloroacetamide | Dinitroanaline | Dinitroanaline |
| **IUPAC Name**[1] | 6-chloro-N-ethyl-N-isopropyl-1,3,5-triazine-2,4-diamine | 2-chloro-6-ethyl-N-(2-methoxy-1-methylethyl)acet-*o*-toluidide | N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine | α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine |
| **Water Solubility, mg/l**[1] | 33 | 488 | 0.3 | 0.22 |
| **Log P ($K_{ow}$)**[1] | 2.5 | 2.9 | 5.2 | 5.3 |
| **$K_{oc}$**[1] | 39-155 | 121-309 | 6,500-29,000 | 6,400-13,400 |
| **Vapor Pressure, mPa**[1] | 0.039 | 4.2 | 4.0 | 9.5 |
| **Year Introduced**[1] | 1958 | 1976 | 1974 | 1963 |
| **Crop Usage**[1] | Corn, sorghum, sugarcane, pasture | Corn, soybeans, sorghum, peanuts | Soybeans, corn, cotton, peanuts, turf | Soybeans, cotton, wheat, sunflowers |
| **Amount applied/ year, million kg – 1987**[2] | 32-35 | 20-23 | 4.5-5.9 | 11-14 |
| **Amount applied/ year, million kg – 1999**[2] | 34-36 | 12-14 | 7.7-10 | 8.2-10 |
| **Soil - Aerobic Half Life**[3]**, Days** | 146 | 26 | 1300 | 169 |
| **Field Dissipation**[3]**, Days** | 173 | 141 | 174 | 81 |
| **Frequency of Detection 1991-2001, % - Stream**[4] | 90 | 83 | 6.6 | 13.3 |
| **Frequency of Detecion 1991-2001, % - Groundwater**[4] | 42 | 17 | 0.28 | 0.69 |
| **Herbicidal Mode of Action**[1] | Inhibits photosynthesis | Inhibits germination | Inhibits cell division | Inhibits cell division |

[1]Reference 18; [2]Reference 19; [3]Reference 20; [4]Reference 21 – data for agricultural sites.

**Organization of Dissertation**

This dissertation is structured as eight chapters – a general introduction, four original research journal articles, two book chapters that review and draw broader conclusions of the research recently conducted in the Pesticide Toxicology Laboratory (Department of Entomology, ISU), and a general conclusion. The first journal article compares the fate of several herbicides in soil columns that are vegetated or left unvegetated. This study treats the column as a system – incorporating dissipation, movement, and bioavailability measurements during one test. The second journal article compares several species of grasses for potential use in vegetative buffer strips. Grassed columns and unvegetated columns were treated with herbicide-fortified artificial runoff. The movement and fate of the herbicides was determined as well as infiltration rates and herbicide degradation potential in the rhizosphere for each species. The third journal article concentrates on pendimethalin; the fate of $^{14}C$-pendimethalin was determined in enclosed chambers with and without the presence of prairie grasses. Measurements included mineralization, volatilization, and uptake into the grass. The fourth journal article continues the study of pendimethalin; the high persistence of some compounds, such as pendimethalin, results in the likelihood that some level of contamination will still be present after remediation attempts. In this manuscript, the toxicity of pendimethalin to soil organisms and the potential effects on birds and rodents as a result of trophic transfer was considered. The results of these experiments are presented as a hazard evaluation of pendimethalin-contaminated soil. For each journal article, I primarily conducted the research and wrote the manuscripts. Additional authors helped in performing tasks related to the research, helped in formulating ideas prior to the study, and/or helped in formulating the research into a manuscript. The first book chapter is an overview of several studies investigating the persistence and bioavailability of dinitroanaline herbicides and the second book chapter is an overview of recent phytoremediation work. Both chapters include original research conducted by me, and also work conducted by others; however, both chapters were entirely written by me, and each includes a new synthesis of the data, intended to provide a more concise and supported description of the research than could be presented by each study individually.

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# CHAPTER 2. EFFECT OF PRAIRIE GRASS ON THE DISSIPATION, MOVEMENT, AND BIOAVAILABILITY OF SELECTED HERBICIDES IN PREPARED SOIL COLUMNS

**Jason B. Belden, Todd A. Phillips, and Joel R. Coats**

**A paper accepted by *Environmental Toxicology and Chemistry***

**Abstract**

Phytoremediation of pesticide-contaminated sites using a prairie grass mixture (big bluestem, yellow indiangrass, and switch grass) has been suggested as a low-cost *in situ* remediation strategy. In this study, the proposed phytoremediation technique was applied to artificially prepared soil columns that were fortified with high concentrations of four herbicides (atrazine, alachlor, metolachlor, and pendimethalin). The fate and toxicity of the herbicides were compared to results from soil columns lacking vegetation. After either 150 or 240 days of phytoremediation, soils were watered with 7.5 cm of water, and leachate was collected. Columns were then divided into three sections (top, middle, bottom). For each section of the column, chemical analysis (ethyl acetate and water extractions), earthworm accumulation tests, and lettuce seedling growth tests were performed. The leachate was chemically analyzed and tested for chronic toxicity to algae. Atrazine and alachlor degraded rapidly in the column, and the total amount recoverable was less than 2% of applied. After 250 days, vegetation reduced the total recoverable amounts of metolachlor and pendimethalin by 78% and 39%, respectively. Metolachlor was the only compound found in leachate, and the amounts recovered were reduced 5 to 20 fold by vegetation. Vegetation decreased the bioavailability of pendimethalin as measured by 8-day, earthworm BAFs (bioaccumulation factors) and lettuce seedling growth assays. Decreases in mobility and bioavailability indicate that this technique may stabilize pesticide residues in addition to increasing dissipation rates.

**Key words**: Phytoremediation, Pendimethalin, Metolachlor, Bioavailability, Soil column

## Introduction

Intensive use of pesticides has resulted in contaminated soils and water systems throughout the agricultural regions of the United States. Initial reports from the National Water Quality Assessment conducted by the United States Geological Survey indicate that 95% of stream and 50% of groundwater samples contained detectable levels of at least one pesticide [1]. When contaminated water is used for drinking or recreational activities, human health may be compromised. Most of the pesticides contaminating water systems were intentionally or incidentally applied to soil, then entered surface water as runoff or leached into groundwater.

Pesticide residues remaining in soil also can result in environmental risk. Pesticides may accumulate in soil organisms, resulting in distribution of the toxicant into the terrestrial food chain, or the pesticide simply could impair the environment by reducing productivity of the immediate area of the contaminated soil. Although standard agricultural pesticide use - which may result in non-point source contamination - often is regarded as the primary source of pesticide contamination, point source contamination originating from agrochemical dealerships, manufacturing and formulation facilities, or spills has also become a serious concern [2, 3]. Current technologies for cleanup of pesticide-contaminated soil often involve excavation, which is disruptive to the environment and very expensive. Phytoremediation has been suggested as a low cost, in situ approach for intermediate contamination problems.

Several recent studies have demonstrated that many plants can increase the dissipation rate of pesticides from soil through uptake into the plant tissue, further degradation in the plant [4], and increased microbial degradation in the rhizosphere [5, 6]. Additionally, vegetation may reduce leaching by decreasing the rate of movement of organic compounds through the soil column [7].

Degradation of organic compounds in the rhizosphere may be a result of cometabolism, where microorganisms coincidentally degrade compounds as a result of enzymes produced during metabolic activity. During degradation processes, metabolic products may be formed before total mineralization of the pesticide occurs. Although often considered to be less toxic, pesticide metabolites also may be a threat to human and

environmental health. Pesticide metabolites are not always measured in drinking or surface water and usually are not considered in evaluating the success of bioremediation techniques.

In a series of studies, the degradation rates of several major herbicides (including metolachlor, atrazine, and trifluralin) have been measured using phytoremediation techniques involving several plant species. These studies have demonstrated that addition of vegetation to pesticide-contaminated soil significantly reduces the amount of pesticides that are extractable. In one experiment, $^{14}$C-atrazine fortified soil was either vegetated with *Kochia* or left unvegetated. After 75 days, *Kochia* vegetated soils had reduced concentrations of $^{14}$C-atrazine compared with nonvegetative soils [8]. In another experiment, the influence of prairie grasses was investigated. Soil fortified with atrazine, metolachlor, trifluralin, and pendimethalin was placed into tubs in a field plot. Each tub was vegetated with a mix of three prairie grasses or left unvegetated. After 19 days of remediation, prairie grass treatments had significantly lower levels of atrazine, metolachlor, and trifluralin compared with soil left unvegetated. No difference was noted in pendimethalin concentrations between treatments [6].

Early successes attained in preliminary tests of phytoremediation of petroleum and industrial organics have rapidly led to implementation of techniques to field trials [9]. However, several important questions regarding the usefulness of phytoremediation, especially for pesticide-contaminated soil, remain unanswered: (1) Are *toxic metabolites* left in the soil? Although mass balance procedures using radiolabeled pesticides are useful in small laboratory tests, conducting such procedures under field conditions is difficult. In field conditions, analysis of metabolites must be done using standard chromatographic techniques that may not provide measurements sensitive and selective enough for all relevant metabolites that could occur during degradation of complex pesticide mixtures. (2) Is rigorous chemical analysis the best endpoint for evaluating remediation success or are *biological endpoints* equally valuable? Biological availability of pesticide residues in soil is usually more relevant to any environmental risk associated with contaminated soil; it also influences biodegradation of pesticides in the soil. (3) During the in situ remediation process, does contamination leave the soil and disperse into critical environmental matrices such as ground and surface water? Do plants reduce the rate of *leaching of contaminants*

down through the soil? Phytoremediation may be a long process, during which it is important that further contamination of nearby areas is not occurring.

In this study, mixed prairie grasses (big bluestem, yellow indiangrass, and switchgrass) were evaluated as potential tools for phytoremediation of atrazine, alachlor, metolachlor, and pendimethalin. These grasses have several attributes that make them good choices for phytoremediation. They are easily attainable as seed, grow quickly, produce deep roots, are considered desirable species, and have been reported to increase dissipation of pesticides [6] and petroleum products [10]. The phytoremediation technique was evaluated in an artificially made soil column, utilizing chemical and biological endpoints to determine the success of the remediation strategy. This evaluation technique provided opportunities for measurement of pesticide movement in the soil column during the remediation process, changes in bioavailability and leaching potential, and the presence of toxic metabolites not measured by the analytical approach. This study had three primary objectives: (1) To determine if the phytoremediation technique increases degradation of the herbicides at various depths in the soil column. (2) To determine if phytoremediation reduces the amount of chemicals that leach from the column during a "rain event." (3) To evaluate whether alternative chemical and biological endpoints - such as water extraction, chronic algal toxicity tests, lettuce growth inhibition, and earthworm uptake - can provide additional relevant information about the success of the remediation strategy.

## Methods

*Preparation of columns*

Soil from a cornfield near Ames, IA, USA that had not received herbicide treatment for over 10 years (Field 55, ISU Ag Engineering/Agronomy Farm) was collected and sieved (2.8 mm) to remove plant material and rocks. Analysis of the soil indicated a sandy loam texture (60% sand, 22% silt, and 18% clay), with 2.7% organic matter, and a pH of 7.0 (Midwest Laboratories, Omaha, Nebraska, USA). The soil was collected as a composite from a site mapped as Nicollett and Webster. The soil was stored at 4°C in polyethylene bags prior to use (less than 90 days). Columns were constructed in polyvinyl chloride pipe (PVC; 10.2 cm in diameter, 23 cm long) enclosed at the bottom with aluminum screen and

glass wool. PVC is a commonly used material for underground water sampling for pesticide analysis and has been previously shown to minimally adsorb most pesticides including atrazine, alachlor, and metolachlor [11, 12].

Due to the length of time necessary to construct columns initially and to process the columns at the end of the study, columns were constructed in four blocks allowing one week between each block. Each block consisted of four herbicide-fortified columns and four unfortified columns. The unfortified columns provided clean soil and leachate for use as controls in the biological assays. Each block served as a single replicate for the 2x2 complete block design. Time (160, 250 days) and presence of vegetation (not vegetated, vegetated) were the treatments studied. Four blocks were constructed (n=4 for each treatment).

Herbicide-fortified columns were packed with 7 cm unfortified soil at the base of the column and topped with 14 cm of soil fortified with atrazine, alachlor, metolachlor, and pendimethalin, each at 25 mg/kg (36.9 mg per column). Soils were treated by adding a herbicide solution in reagent grade acetone at a rate of 5 ml per 300 g of soil. Soil was mixed thoroughly on unbleached paper 20 times and then left open on a counter for 6 hours to allow the acetone to evaporate. The fortified soil was then added to 700g of unfortified soil in a 4-L amber jar (1 kg soil). The jar was then rotated for 4 hours at a rate of 30 rpm. Columns were packed to a bulk density of 1.25 g/cc. Unfortified columns were constructed in an identical manner except that only the reagent grade acetone was used instead of the herbicide solution. Distilled water was added slowly (1 cm /hour) to each column to reach a moisture content of 1/3 bar (19.1%). Columns were aged for 60 days at greenhouse conditions (25° C ± 3° C, 16:8 minimum light to dark). During aging, the columns were watered with 1 cm (81 ml) of distilled water every 96 hours. This amount kept the columns moist yet was insufficient to cause loss of water from the bottom of the column.

*Phytoremediation of columns*

Big bluestem (*Andropogon gerardii*), yellow indiangrass (*Sorghastrum nutans*), and switchgrass (*Panicum virgatum*) seed were purchased from United Seed Company (Omaha, NE, USA). Plugs of each type of prairie grass were obtained by planting 10 seeds in a small cone of potting soil (5 g). Within two weeks of sprouting, one plug of each grass type was added to 8 herbicide-fortified columns and 8 unfortified columns. The remaining 16 columns

received plugs of potting soil only. Columns were kept under the same greenhouse conditions and on the watering schedule discussed above.

*Leaching of soil columns*

After 150 days of remediation (or 240 days for the second time point), 7.5 cm (608 ml) very soft water (12.0 mg/L as $NaHCO_3$) was added to each column over an 8-hr period. Leachate was collected at the bottom of the column. Leachate was stored at 4°C for no longer than 48 hours before use in algal bioassays and solid-phase extraction.

*Removal of soil from the columns*

For 10 days after leaching (until 160 days of remediation for time point 1, and 250 days for time point 2), the columns were left under greenhouse conditions without water. Vegetation was then trimmed from the top of each soil column, and the soil from each column was collected from three regions - the top 6 cm, the following 6 cm (middle), and the bottom 5 cm. The soil was sieved (2.8 mm), vegetation was removed (except for fine roots able to pass through the sieve), and the soil was stored in glass containers at 4°C. Sections of each column were treated statistically as split plots. One aliquot of soil from each split plot (each section of each column) was used for ethyl acetate extraction, water extraction, earthworm uptake, and lettuce seedling growth studies.

*Soil extraction with ethyl acetate*

Twenty grams (dry mass) of each soil sample was extracted 3 times with 60 ml of ethyl acetate. The extract was filtered and concentrated to 10 ml. Analysis was performed using a Varian 3400 Gas Chromatograph equipped with a thermoionic specific detector (nitrogen and phosphorus specific; GC-TSD; Walnut Creek, CA, USA). Extraction efficiency for this method was determined by treating four aliquots of sieved soil with each herbicide using acetone as a carrier. The spiking solution was added directly to the soil followed my mixing to allow evaporation of the acetone. Recoveries were 91% for atrazine with a standard deviation of 8 (SD 8), 85% (SD 10) for deethylatrazine, 87% (SD 9) for deisopropylatrazine, 95% (SD 8) for alachlor, 103% (SD 7) for metolachlor, and 95% (SD 11) for pendimethalin. Prior to starting the test, soil from each block was analyzed to insure that fortification levels were correct. Each pesticide in every sample was within 89-104% of the target value.

*Extraction of leachate*

Extraction of water samples was performed by solid-phase extraction followed by analysis using gas chromatography. Solid-phase extraction tubes (SPE, Envi-18, Supelco, Bellefonte, PA, USA) were sequentially conditioned with ethyl acetate (3 ml), methanol (3 ml), and water (5 ml). Leachate (100 ml) was passed through the tubes (10 ml / min), and the tubes were allowed to air-dry for 4 minutes. Three 4-ml aliquots of acetone were then used to elute herbicides off the SPE tube. The extract was concentrated to 5 ml and analyzed by GC-TSD. Extraction efficiencies for this method, determined from freshly fortified water, were 96 % (SD 6) for atrazine, 87 % (SD 11) for deethylatrazine, 75 % (SD 14) for deisopropylatrazine, 92 % (SD 6) for alachlor, 94 % (SD 5) for metolachlor, and 88 % (SD 3) for pendimethalin.

*Soil extraction with water*

Ten grams (dry wt.) of each sample was extracted 3 times with 30 ml of soft water (12 mg/L $NaHCO_3$). The aqueous extract was centrifuged for 20 minutes to remove suspended solids. The aqueous extract was analyzed by SPE and GC-TSD as described above.

*Earthworm Bioassay*

Eight-day earthworm bioaccumulation assays were conducted similarly to previously reported methods [13]. These assays were conducted as a measure of bioavailability. Soil from each column section was adjusted to 19% moisture (approximately 1/3 bar), and 150 g was placed in 200-ml jars. Four earthworms (*Eisenia fetida*) totaling approximately 1.5 g, were placed in each of the labeled jars and sealed with perforated Parafilm®. Jars were incubated at 25°C for 8 days. The earthworms were removed from sample soil and placed in untreated soil for one day. After 24 hours in untreated soil, the earthworms were removed and stored at -60°C. They were extracted 3 times with 10 ml of ethyl acetate in a sample homogenizer. The extract was filtered, concentrated to 5 ml, and analyzed using GC-TSD as previously described. Biological accumulation factors (BAFs) were calculated as (1) concentration in the earthworm/concentration recoverable by ethyl acetate extraction (BAF-solvent) or (2) concentration in the earthworm/concentration recoverable by water extraction (BAF-water). Identical studies were performed using soil from the same source with worms

added immediately after fortifying the soil with pendimethalin. These additional studies provided a baseline for the theoretical (expected) BAF for pendimethalin.

*Lettuce seedling growth*

Soil from each column section was adjusted to 19% moisture (1/3 bar), and 125 g were placed in 200-ml jars. Ten lettuce (*Lactuca sativa*) seeds (Carolina Biological, Burlington, NC, USA) were added to each jar and covered with plastic wrap having pin-sized ventilation holes. Jars were incubated at 20°C with a 16:8 light:dark cycle (3500 lux) for 7 days. Lettuce seedling growth was measured by recording the total length of germinated seedlings in each jar. Percentage inhibition was measured by comparing growth in each section of the herbicide-fortified soil columns to that found in corresponding unfortified soil columns. Reference toxicity tests (conducted with the same conditions except using fresh pendimethalin residues) were conducted. After log transformation of the concentration, pendimethalin had a linear ($r^2 = 0.97$) relationship for inhibition within the range of 15 to 85%. The resulting equation was used to estimate an available concentration in the sample based upon the percentage inhibition that each bioassay yielded. Ratios of the estimated available concentration divided by the concentration determined by rigorous solvent extraction were calculated as a measurement of bioavailability.

*Algae growth*

Dilutions up to 16x were made for each leachate sample, by factors of 2. Three random wells in a polystyrene 96-well microplate were filled with 0.125 ml of each leachate/dilution. Additionally, 0.100 ml of a buffer and nutrient solution (Alga-gro, Carolina Biological, Burlington, NC, USA - diluted 1:40 in hard water, 196 mg/L $NaHCO_3$) and 0.025 ml of freshly cultured *Selenastum capricornutum* were added, resulting in an original algal cell count of 50,000 cells/ml in each well. Microplates were incubated for 96 hours at 25°C and 4000 lux of continuous soft fluorescent light. Absorbance readings were made at 450 nm on a microplate reader using blank measurements from each treatment/dilution for absorbance correction. Percent inhibition was measured by comparing the absorbance value for each leachate/dilution obtained from herbicide-fortified column to the value from the corresponding unfortified column. Concentration of the herbicides at the dilution estimated to cause 50% effect was determined and compared to EC50 values previously determined for each herbicide.

*Statistics*

The experiment was set up in a randomized complete block, with length of remediation time and presence of vegetation as treatment factors and each replicate as a block. Whole column measurements (pesticide mass recoverable from leachate and total column) were analyzed using analysis of variance (ANOVA, PROC GLM). Additional analyses compared columns using measurements taken from each section. Each section was a subunit of the experimental unit (column), resulting in the use of a split-plot analysis of variance analysis (PROC MIX). Mean separation tests were conducted using Least-square means (LSMEANS). Determination of difference of treatments from the control for bioassays was accomplished using Dunnett's test for significance. All calculations were conducted using SAS [14].

**Results**

*Phytoremediation of columns*

All vegetated columns were successful in growth of grass. The average dry biomass obtained at 160 days was 4.8 g (SE 0.9 g), while the average dry biomass obtained by 250 days was 9.0 g (SE 2 g). Due to evapotranspiration, the percentage of water obtained during the leaching event was significantly different between vegetated and unvegetated columns ($F=51$, $p<0.001$). Vegetated columns only allowed 41% of the added water to move through the column, while 60% moved through the unvegetated column.

*Chemical analysis of leachate and soil by ethyl acetate extraction*

Atrazine and alachlor degraded rapidly. Alachlor was not found above the detection limits of the analytical methods (less than 0.5 % of applied) in any soil column sample or in the leachate, or for either time point. Atrazine and primary atrazine metabolites, deethylatrazine (DEA) and deisopropylatrazine (DIA), were found in the bottom section of the column and in leachate samples. However, no significant differences were observed between treatments, and residues accounted for less than 2% of applied atrazine.

The total amount of metolachlor recovered (from the column and leachate combined) was significantly less in vegetated compared with unvegetated columns ($p<0.001$) and in columns measured at 250 days compared to 160 days ($p=0.002$, Table 1). Comparison of metolachlor recoveries throughout sections of the column indicate that presence of vegetation

($p$=0.017), length of remediation ($p$<0.001), and section ($p$<0.001) all had a significant effect on the metolachlor concentration. Significant interaction was observed between section and length of remediation ($p$<0.001) and between section and presence of vegetation ($p$=0.036). In both instances, the effect of the treatment (vegetation or remediation length) was less for the top section than for the lower sections. Within the column, highest metolachlor concentrations were found in the bottom of the column (unfortified portion) and in leachate. The amount of metolachlor in the leachate was significantly less in vegetated as compared

Table 1. Distribution of herbicides throughout soil columns as measured by solvent extraction. Average amounts represented as percentage of applied are shown with the standard error in parenthesis (n=4).

| | | 160 Days, % of Applied | | 250 Days, % of Applied | |
|---|---|---|---|---|---|
| Compound | Section | Unvegetated | Vegetated | Unvegetated | Vegetated |
| Metolachlor | Top[a] | 1.0 (0.1) | 0.74 (.24) | 1.1 (0.2) | 0.50 (0.24) |
| | Middle[a] | 5.0 (0.3) | 2.6 (1.0) | 2.3 (0.5) | 0.64 (0.21) |
| | Bottom[a] | 9.6 (0.9) | 6.0 (1.2) | 2.8 (1.5) | 0.51 (0.22) |
| | Leachate[b] | 7.6 (1.3) | 1.5 (0.3) | 1.5 (0.6) | 0.08 (0.03) |
| | Total[c] | 23 (2) | 11 (2) | 7.8 (2.1) | 1.7 (0.5) |
| Pendimethalin | Top[d] | 16 (1) | 11 (1) | 11 (1) | 8.0 (0.5) |
| | Middle[d] | 25 (1) | 17 (1) | 19 (2) | 9.9 (0.8) |
| | Bottom[d] | 0.15 (0.07) | 0.35 (0.14) | 1.1 (0.7) | 0.52 (0.28) |
| | Leachate | <0.05 | <0.05 | <0.05 | <0.05 |
| | Total[e] | 41 (2) | 28 (6) | 31 (2) | 19 (3) |

Section (F=36.3, $p$<0.001), presence of vegetation (F=23.6, p=0.017), and length of remediation (F=58.3, $p$<0.001) had significant effects on metolachlor concentrations.

a) Presence of vegetation (F=26.0, $p$< 0.001) and length of remediation (F=26.0, $p$<0.001) had a significant effect on the amount of metolachlor recovered from leachate.
b) Presence of vegetation (F=16.0, $p$<0.001) and length of remediation (F=48.6, $p$<0.001) had a significant effect on total amount of metolachlor recoverable.
c) Section (F=221, $p$<0.001), presence of vegetation (F=31.2, $p$=0.011), and length of remediation (F=20.5, $p$=0.004 ) had significant effects on pendimethalin concentrations.
d) Presence of vegetation (F=26.6, $p$<0.001) and length of remediation (F=16.3, $p$<0.001) had a significant effect on total amount of pendimethalin recoverable.

with unvegetated columns ($p<0.001$) and for 250 days as compared with 160 days ($p<0.001$; Table 1). Significant interaction was observed between vegetation and time ($p=0.008$). Time had less effect on unvegetated columns compared with vegetated columns.

The total amount of recovered pendimethalin (from the leachate and the column combined) also was significantly reduced in vegetated columns compared with unvegetated ($p<0.001$) and was reduced in columns remediated for 250 days compared with 160 days ($p=0.002$, Table 1). Analysis of the columns by section indicated that pendimethalin recovery was affected significantly by length of remediation ($p=0.004$), presence of vegetation ($p=0.011$), and section ($p<0.001$). As with metolachlor, significant interaction was observed between length of remediation and section ($p=0.007$) and between vegetation and section ($p<0.001$). The effect of time and vegetation had a greater magnitude in the middle section than in the top section. Table 1 shows the distribution of pendimethalin in the columns. One percent or less of the applied pendimethalin was recovered from the bottom of the columns. Pendimethalin was not found in any leachate samples (<0.05% of applied).

*Chemical analysis of soil by water extraction*

Water extraction of soil yielded trends for metolachlor recovery similar to those of solvent extractions. Increased length of remediation ($F=15.9$, $p=0.007$) and presence of vegetation ($F=9.57$, $p=0.054$) decreased recoverable amounts of metolachlor, while lower sections of the soil column had greater concentrations than the upper sections ($F=41.0$, $p<0.001$, data not shown). The amount of metolachlor that was water-extractable per solvent-extractable is shown in Figure 1. The ratios were not significantly affected by the presence of vegetation or by the length of remediation. However, metolachlor in the bottom sections of the column was more efficiently extracted with water than the middle and the top sections ($p<0.05$).

The amount of pendimethalin recoverable from the soil by water extraction also followed a similar trend to that noted for solvent extractions. Length of remediation ($F=42.1$, $p<0.001$), presence of vegetation ($F=9.08$, $p=0.057$), and section ($F=158$, $p<0.001$) all had significant effects (data not shown). Comparison of the amount of pendimethalin that was water extractable per solvent extractable indicated that the longer the length of remediation, the lower the percentage that was water extractable ($p=0.014$) and that the top section yielded

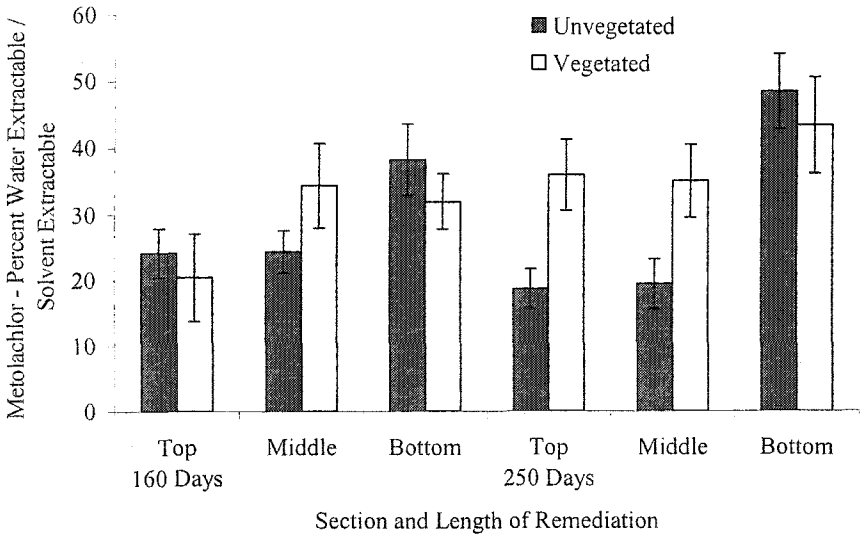


Figure 1. Mean percentage ratios of metolachlor amounts that were extractable by water divided by the amounts that were extractable by solvent in each section of vegetated and unvegetated columns (n=4). Only section showed a significant effect (F=14.7, $p< 0.001$). Error bars illustrate one standard error.

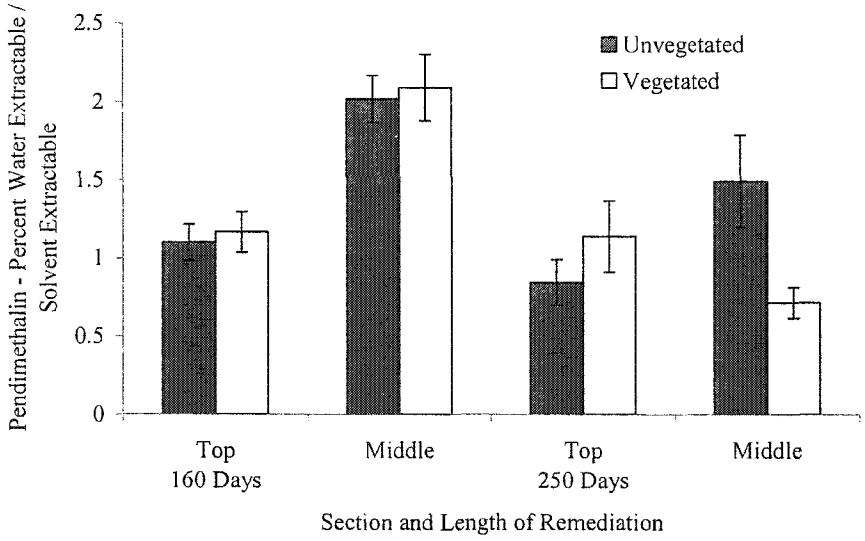


Figure 2. Mean percentage ratios of pendimethalin amounts extractable by water divided by the amounts extractable by solvent in the top two sections of vegetated and unvegetated columns (n=4). Pendimethalin concentrations were below detection limits for all bottom sections. Length of remediation (F=12, $p$=0.014) and section (F=69, $p$<0.001) both had significant effects on ratio. Error bars illustrate one standard error.

lower water extraction percentages compared with the middle section ($p<0.001$; Figure 2). Pendimethalin was not detected in water extraction samples from the bottom section of the column. The presence of vegetation did not significantly influence the percentage of pendimethalin that was water extractable.

*Earthworm uptake*

Pendimethalin was the only herbicide detectable within earthworm bodies, and it was only found for worms exposed to the top and middle column sections. For assessment of changes in bioavailability, BAFs were calculated using concentrations measured in the soil by solvent extraction (solvent -BAF) and water extraction (water-BAF). The presence of vegetation ($p=0.034$) and longer lengths of remediation ($p<0.001$) reduced solvent-BAFs (Figure 3). The top sections of the column had significantly lower solvent-BAFs than did the middle section ($p<0.001$). Calculations using water extraction data also showed a significant reduction of water-BAFs by an increased length of remediation ($p=0.008$), as well as a consistent trend in lower BAFs due to presence of vegetation, although the difference was not significant ($p=0.127$, Figure 4). Significant interaction existed for water-BAF between section and length of remediation ($p<0.001$). At 160 days, the middle section tended to have lower BAF values; while at 250 days, the top section tended to have lower BAF values. Further statistical analysis indicates that at each time point, differences existed between the two sections ($p< 0.05$).

*Lettuce seedling growth*

Soil samples from the top and middle sections after 160 days of remediation inhibited lettuce seedling growth, whereas the bottom section at 160 days and all samples after 250 days of remediation exhibited less than 13% inhibition and were not significantly different from the control ($p<0.05$). At the first time point, lettuce seedlings grown in the top section of unvegetated columns were inhibited 49% (SE 7) and in the middle section were inhibited 52% (SE 5). The presence of vegetation significantly reduced inhibition ($F=20.7$; $p=0.001$), compared with unvegetated controls. Lettuce seedlings grown in the top section of vegetated columns were inhibited by 23% (SE 5), while the middle section was inhibited by 35% (SE 4). Length of remediation also significantly reduced toxicity ($F=7.26$, $p=0.036$). Section did not have a significant effect on toxicity.

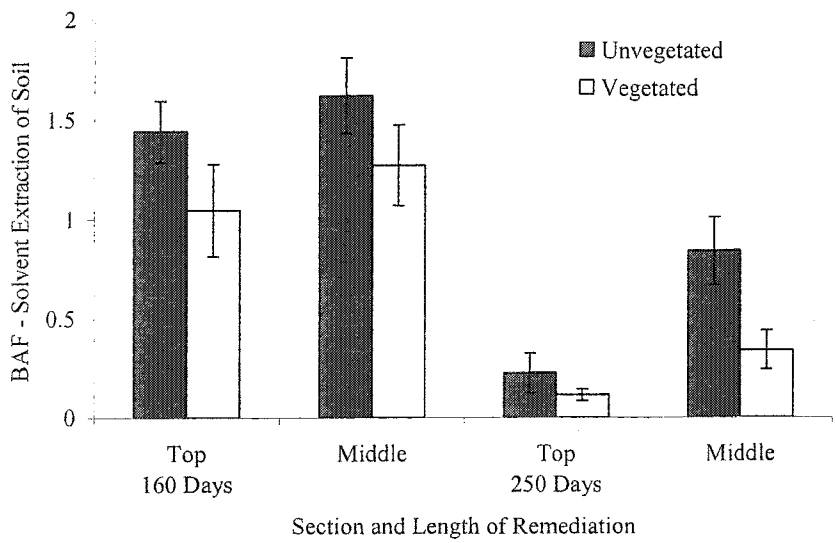


Figure 3. Mean eight-day earthworm bioaccumulation factors (BAF) based on soil concentration determined by solvent extraction (n=4). Error bars indicate one standard error. Presence of vegetation (F=7.5, $p$=0.034), length of remediation (F=32, $p$<0.001), and section (F=29, $p$<0.001) had a significant effect on BAF .

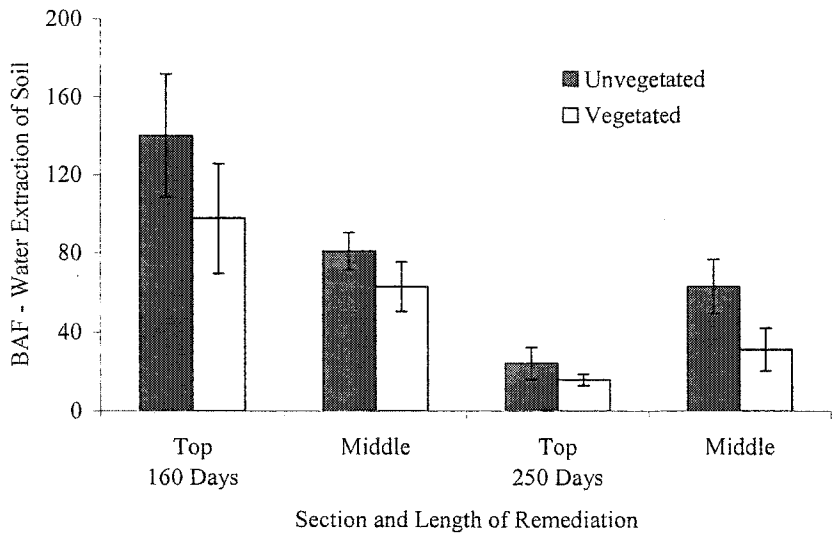


Figure 4. Mean eight-day earthworm bioaccumulation factors (BAF) based on soil concentration determined by water extraction (n=4). Error bars indicate one standard error. Length of remediation (F=15, $p$=0.008) had a significant effect on BAF values based on water extraction. In addition, a significant interaction was present between length of remediation and section ($p$=0.007).

Ratios of the concentration, as determined by lettuce toxicity testing, divided by the concentrations, as determined by ethyl acetate extraction, were calculated for each soil sample. Since samples from the bottom section after 160 days of remediation and all samples after 250 day of remediation inhibited seedling growth less than 15%, the ratio should be considered an estimate because the toxicity data was not in the linear range of the bioassay to pendimethalin concentration conversion. As shown in Figure 5, the pendimethalin concentration predicted by bioassay for unvegetated columns after 160 days of

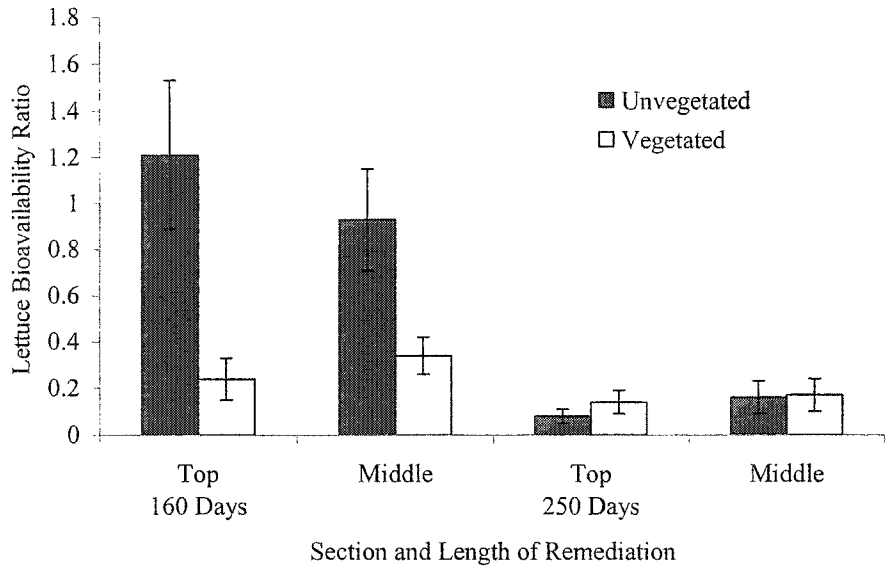


Figure 5. Mean ratios of the expected concentration of pendimethalin based on observed toxicity to lettuce and the concentration determined by rigorous solvent extraction was used as an indicator of bioavailability (n=4). Length of remediation significantly reduced the ratio ($F=8.7$, $p=0.026$), as did presence of vegetation at 150 days ($F=13$, $p=0.006$).

remediation, corresponded well with the concentration determined by solvent extraction. The presence of vegetation significantly reduced the ratio ($p=0.006$), indicating a drop in bioavailability. The bioavailable fraction of pendimethalin also was significantly reduced by length of remediation ($p=0.026$).

*Algae toxicity tests*

The dilution estimated to cause 50% inhibition was determined for each leachate sample (inhibition dilution factor, IDF50). If a 16x dilution was insufficient to reduce toxicity to 50% effect, 16 was used as the IDF50; likewise, if 1x was insufficient to cause 50% effect, 1 was used as the IDF50. Length of remediation ($p<0.001$) and presence of vegetation ($p<0.001$) significantly reduced the IDF50. After 150 days of remediation, leachate from unvegetated columns had a mean IDF50 of 14 (SE 1), whereas vegetated columns had a mean IDF50 of 5.3 (SE 1.6). After 240 days of remediation, leachate from unvegetated columns had mean IDF50 values of 6.5 (SE 1.9), whereas vegetated columns had mean IDF50 of 2.0 (SE 0.6). In addition, measured concentrations of metolachlor for each leachate sample were used to determine the metolachlor concentration corresponding to each IDF50. This value is, in effect, an IC50. Comparison of these metolachlor concentrations between treatments indicated no significant differences for length of remediation or presence of vegetation. The average concentration at the IDF50 for all samples was 110 μg/L. The IC50 (inhibition concentration) of metolachlor was determined to be 90 μg/L (95% confidence intervals were 72-108 μg/L).

**Discussion**

Dissipation of atrazine and alachlor proceeded rapidly in the moist, warm soil conditions set up for the testing. The persistence of both compounds has been shown to be quite variable depending on an array of environmental factors including concentration [15, 16]. For instance, alachlor applied to a clay loam soil at different concentrations degraded to 1.9% of applied after 140 days when a 10 mg/kg application rate was used, yet 63% remained after 140 days when a 1000 mg/kg rate was used [15]. In a similar study, atrazine applied to a sandy loam soil degraded to 6.3 % of applied when a 5 mg/kg application rate was used, yet 41% remained after 140 days when a 5000 mg/kg rate was used [16]. In the same study, atrazine was applied to a clay loam soil resulting in less than 1.8% of applied remaining after 140 days, regardless of the applied concentration. In this study, conditions resulted in fast dissipation of these herbicides; however, aged residues of atrazine and alachlor have been reported to exist at agrochemical dealerships [3], likely due to large initial concentrations resulting in slower degradation kinetics. Although information regarding this

phytoremediation strategy is not available from this study, both compounds are potential candidates for bioremediation approaches.

This study confirms previous work that indicates prairie grasses can cause an overall increase in metolachlor dissipation [6]. As shown in Table 1, the total amount of metolachlor recovered after 250 days of vegetation was reduced by 78% of the amount recovered in unvegetated column. The amount of metolachlor recoverable in the vegetated columns was less than 2% of applied. Previous short-term studies have not found significant increases in pendimethalin dissipation with the addition of prairie grasses [6]. But, in the current study, pendimethalin concentrations were reduced significantly by vegetation after 160 days and the trend continued to 250 days, with vegetated columns having 39% less pendimethalin than unvegetated columns (Table 1). Although the remediation technique increased pendimethalin dissipation, 19% of the applied pesticide still remained, indicating that longer remediation may be necessary.

Metolachlor readily moved throughout the soil column during the remediation period and the "rain event." The majority of recovered metolachlor was found in the bottom section or in the leachate (Table 1). Pendimethalin did not move through the soil column to any extent. The majority of recoverable pendimethalin was obtained from the top two sections where it was applied (Table 1). Previous studies have reported similar trends in mobility. Metolachlor previously has been reported to leach extensively through intact soil columns growing corn [17], whereas pendimethalin has been reported to stay within the top 9 cm of the soil column when freshly applied and has even less migration potential a few days after application, due to low desorption rates from soil [18].

The presence of prairie grasses in the column not only decreased the overall amount of metolachlor recovered, it also decreased the percentage of remaining metolachlor that entered the leachate during the "rain event" (Table 1). Metolachlor recoveries in leachate from columns remediated for 160 days represented 33% of recovered metolachlor for unvegetated columns and only 14% for vegetated columns. Columns remediated for 250 days showed a similar pattern, with metolachlor recovered from leachate representing 19% of the total for unvegetated columns and 4.7% of the total for vegetated columns. The reduction may be partially the result of the decreased volume of leachate passing through vegetated columns as a result of evapotranspiration drying the vegetated columns. However, the difference in

volume of recovered leachate was less than a factor of 1.5, while the percentage of metolachlor recovered in leachate differed by a factor of greater than 2 at 160 days and a factor of nearly 5 at 250 days.

The increase in stabilization of the residues may be due to a variety of factors: the vegetation may be increasing the organic matter content of the soil, thereby increasing binding sites; increased microbial growth in the rhizosphere may be increasing the rate of organic matter turnover in the soil, increasing binding potential; or micropores around plant roots may allow for preferential flow through the column, allowing water to drain without seeping through the entire soil column containing the herbicides. Previous studies have shown that earthworm burrows decreased the amount of atrazine that leaches from a soil column after atrazine is distributed throughout the soil [19].

The percentage of water-extractable herbicide compared with solvent-extractable herbicide was increased for pendimethalin in the middle level and for metolachlor at the middle and lowest levels in the soil columns (Figures 1 and 2). This trend showed an increased availability of the herbicide for leaching and possible uptake into biota. Lower levels of availability in the upper column may be indicative of leaching of available herbicide residues to the lower level, leaving less available or bound fractions behind. The top of the column also had an increased level of organic matter compared with the rest of the column, due to the potting soil that was added directly to unvegetated columns or with the plants in vegetated columns. Presence of vegetation did not significantly affect water/solvent extraction ratios for metolachlor, but significant differences were found for pendimethalin. Pendimethalin has a higher affinity for binding to organic matter and has a lower water solubility compared with metolachlor and thus may be more easily retained due to changes in organic matter that resulted from increased plant or microbial activity.

Changes in pendimethalin bioavailability were apparent in both the earthworm uptake studies and the lettuce seedling growth assays. As shown in Figures 3 and 5, longer periods of remediation and presence of vegetation reduced pendimethalin uptake into earthworms and reduced the observed toxicity to lettuce. This indicates that concentrations of pendimethalin obtained by rigorous solvent extraction are likely to overestimate the risk of aged residues. Other studies have shown similar trends for persistent organic compounds [20]. Mechanisms of decreased bioavailability are likely similar to those discussed for

increased stabilization of metolachlor leaching. Increased amounts or turnover of organic carbon in the soil could result in greater binding potential. An additional likely mechanism is that the moisture balance was different in vegetated columns versus the unvegetated columns. Evapotranspiration of water by the plants caused a greater degree of drying cycles within the column. Pendimethalin has been shown to have reduced mobility when added to dry columns compared with moist columns [18]. Additional work has shown that phenanthrene is sequestered to a greater extent if wetting and drying cycles occur compared with constant soil moisture [21].

Both lettuce and algal bioassays indicated that the toxicity expected from measurement of the chemical concentration was at the same level or higher than the toxicity actually found in the bioassay. These results are important because they suggest the metabolites produced are either not phytotoxic or exist at concentrations below which toxicity occurs. In the absence of measuring all metabolites present during remediation and their toxicity, bioassays can provide important measurements for monitoring the success of a remediation project.

Although several field studies investigating phytoremediation of organics have been started in the past few years [22], few studies have investigated phytoremediation techniques in controlled environments in which knowledge of mobility and degradation processes can be measured [9]. This study demonstrates that prairie grasses may help mitigate pesticide-contaminated sites not only by increased degradation rates but also by stabilizing pesticide residues while phytoremediation is occurring or while the site is being studied for potential remediation. Reduced leaching is important because most bioremediation techniques will not completely eliminate all pesticide residues that are likely to be found at an agrochemical dealership site. Bioassay techniques such as those used in this study are important in estimating environmental risk that a site may pose, especially since rigorous solvent extraction may overestimate the available fraction of the herbicides.

**Acknowledgement**

Partial financial support for this project was provided by the Center for Health Effects of Environmental Contaminants (CHEEC) at the University of Iowa. This manuscript is publication No. J-19905 of the Iowa Agriculture and Home Economics Experiment Station, Project 3187. Partial funding for J. B. Belden was provided through a Biotechnology

Graduate Fellowship from the Office of Biotechnology, Iowa State University, Ames, IA, USA.

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# CHAPTER 3. COMPARISON OF GRASS SPECIES FOR USE AS PESTICIDE MITIGATING AGENTS IN VEGETATED FILTER STRIPS

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**A paper submitted to *Environmental Toxicology and Chemistry***

## Abstract

Intact soil columns were collected and vegetated with different grass species, a mixture of grasses, or left unvegetated. After a year of growth, artificial runoff containing atrazine, metolachlor, and pendimethalin was applied to the columns followed by additional runoff events weekly for four weeks. Measured endpoints included: time needed for infiltration; amount of each herbicide to leach; amount of herbicide remaining in each column; and rhizosphere activity as measured by DMSO reduction, atrazine mineralization, and formation of metolachlor bound residues. All vegetated treatments increased the infiltration rate of the runoff. Neither type nor presence of vegetation caused a significant change in the amount of total herbicide leaching through the column or total amount of recoverable herbicide. An average of 25% of applied atrazine and 24% of applied metolachlor was recovered from leachate. Pendimethalin was not detected in leachate (<0.18% of applied). However, vegetation decreased the amount of herbicide leached after the initial addition of herbicide to the column. Soil microbial activity was also greater in vegetated columns as demonstrated by increased mineralization of atrazine, increased formation of metolachlor bound residues, and increased DMSO reduction. Overall, mixed prairie grasses, switchgrass, and brome all performed well for most endpoints. Fescue increased infiltration, but otherwise performed similarly to unvegetated columns.

**Keywords:** vegetated filter strip, atrazine, metolachlor, pendimethalin

## Introduction

In the agricultural regions of the United States, pesticide contamination of surface water samples threatens drinking water sources and environmental health. A recent study by the

United States Geological Survey reported the presence of pesticides in more than 95% of surface water samples [1]. Due to their high usage and high water solubility, herbicides were the most common class of pesticides found. Atrazine, 6-chloro-N-ethyl-N-isopropyl-1,3,5-triazine-2,4-diamine, and metolachlor, 2-chloro-6-ethyl-N-(2-methoxy-1-methylethyl)acet-*o*-toluidide, were two of the most commonly found compounds. In agricultural settings, atrazine was detected in 85% of samples and metolachlor was detected in 78% of samples. Both herbicides are used heavily in corn/soybean rotation systems, are relatively water soluble, and are moderately persistent in water. Pendimethalin, N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine, another compound commonly used in corn/soybean systems, was reported in 10% of samples. Although pendimethalin has very low solubility and minimal mobility in the soil column [2], it is considered by the Environmental Protection Agency to be a persistent, bioaccumulative, and toxic compound (PBT) [3] and is, therefore, of interest.

Although several studies have demonstrated the use of vegetative filter strips (VFS) for decreasing the nutrient load and sediment in surface runoff, as well as providing habitat for wildlife [4, 5], fewer studies have been conducted on the use of buffer strips for pesticide load reduction [6]. The studies that have been conducted indicate that VFS do reduce pesticide loads [6-14]. Reduction for weakly sorbed pesticides is mainly due to infiltration of runoff [6, 8]. For example, in a study conducted at the Swine Nutrition Center at Iowa State University, VFS, consisting mainly of smooth bromegrass, had an average infiltration of 59% of surface water, resulting in removal rates of atrazine and metolachlor of 61% and 63%, respectively [6]. Reduction of more strongly sorbed pesticides by VFS may also be achieved by adsorption to vegetation and soil within the VFS [14].

Several factors can influence the efficacy of VFS including soil type, slope, length, vegetation density, and vegetation type [14]. Most VFS research has been conducted using field studies. Due to the large-scale design of such studies, only one or two treatments were evaluated [6, 8-14]. Often the only comparison was made from the chemical load in runoff before entering the filter strip and chemical load leaving the filter strip. In some studies, slope, length, height of grass, soil moisture, and farming practices have been investigated as factors regarding the efficacy of vegetative filter strips [14]. However, little information is available regarding the influence of vegetation type on VHS performance. Although grasses are commonly used, the choice of a certain grass is rarely justified by research findings.

Measurements of soil concentrations of pesticides within the filter strip and vertical movement in the soil profile have rarely been reported [6, 8-14]. A few studies have been conducted on tilted platforms under greenhouse conditions [7]. These studies allowed better control of the experimental conditions and mass balance of pesticide concentrations. However, the tilted bed method réquires artificial preparation of the soil column into a bed, and generally few treatments are used due to the large size required and limitations of resources.

In the current study, we used intact soil columns to evaluate the effect of different grass types on the infiltration rate of water, the movement of atrazine, metolachlor, and pendimethalin that has entered the soil column through infiltration of surface runoff, and the ability of differently vegetated soil to degrade metolachlor and atrazine. By conducting this study as a microcosm experiment using relatively small soil columns (10-cm diameter), five grass types and an unvegetated treatment were simultaneously compared.

## Methods

*Preparation of grassed columns*

Sections of polyvinyl chloride (PVC) pipe, 10 cm in diameter by 30 cm in length, were driven into soil manually using a modified fence-post driver. The soil columns were collected when the soil was slightly drier than field capacity (based upon field conditions, 16% water/dry mass of soil). Wetting of the columns to field capacity allowed some swelling and compression to the sides of the walls, decreasing the potential for edge effects. Soil structure was mostly retained as indicated by the visual examination of macropores on the bottom of the column. The collection technique should provide more realistic results than artificially prepared soil columns, while still being easily obtainable. Thirty experimental units were obtained using this technique. All columns were collected in May, 2001 on the Iowa State University Campus, between 7 and 10 meters from a cornfield that had received regular atrazine and metolachlor applications. The plot was originally vegetated with mostly bluegrass. Sod was removed with a shovel before driving of PVC pipe to collect the columns. Soil analysis, conducted by a contract laboratory (Mid Continent Laboratories, Omaha, NE, USA) classified the soil texture as loam (44 % sand, 32 % silt, and 24% clay), field holding capacity of 21%, and an organic matter content of 2.7 %.

Phosphorus and nitrogen levels were sufficiently at or above recommended levels for prairie grasses, so fertilization was not necessary (167 mg/kg N; 81 mg/kg weak bray P). All columns readily allowed water to drain, after a slight delay, at the time of collection.

Thirty days after collection, columns were seeded with each grass type (see Table 1) or left unvegetated. The seeding rate was 20 seeds per column, which corresponded to approximately 5 kg/ha. The resulting method design included six treatments, five replicates each, completely randomized throughout each step of the experiment. Columns were maintained at greenhouse conditions (approx. 25° C, 16:8 light:dark) with frequent watering. After 210 days of growth in the greenhouse, the columns were placed in an environmental chamber in which the temperature was reduced to 1° C, light was reduced to 10:14 light:dark, and watering was reduced to once every 5 days to allow grasses to senesce. Ninety days later (300 days post-plant), the columns were returned to the greenhouse conditions. All vegetated columns had vigorous new growth. Columns were maintained for 60 days prior to starting the leaching studies (360 days post-plant). Four days prior to receiving artificial runoff, all columns were saturated by watering heavily from above and then allowed to stand while drainage and evaporation of water occurred.

*Infiltration of artificial runoff*

Artificial runoff samples were synthesized based on information obtained from runoff events reported in a previous study conducted at Iowa State University at the Swine Research Center (Ames, IA, USA) [6]. In that study, field plots of corn under traditional tillage practices were treated with atrazine at 2.2 kg/ha and metolachlor at 2.8 kg/ha. Runoff from plots fed into brome VFS at a 30:1 ratio. Five to ten runoff events were recorded each year, with most occurring in the spring. Events close to application time were the primary carriers of the herbicides. Average runoff loss from the field was 4.9 mm depth from source. Herbicide retention in the VFS ranged from 11-100 %, infiltration rates averaged 58 %, and herbicide losses from the field were around 2.5 %.

Using this data, we developed a four-week application of artificial runoff, consisting of one runoff event per week over a four-week period. The amount of herbicide added was designed to reflect high but realistic losses from the field. Studying the worst-case scenario

Table 1. Types of grass used to create vegetated soil columns.

| Common Name[a] | Genus Species | Variety |
|---|---|---|
| Smooth Brome (Brome) | *Bromus inermis* | Northern type |
| Big Bluestem (Big Blue) | *Andropogon gerardii* | Pawnee |
| Tall Fescue (Fescue) | *Festuca arundinacea* | K-31 |
| Switchgrass (Switch) | *Panicum virgatum* | Pathfinder |
| Yellow Indiangrass[b] | *Sorghastrum nutans* | Holt |

a. Name in parenthesis is used in Tables and Figures in the Results section.
b. Yellow Indiangrass was planted with Switchgrass and Big Bluestem in the mixed prairie grasses treatment (Mixed).

allows evaluation of the grass types when the potential environmental impact is the greatest. Artificial runoff amounts were based on the scenario of weekly rain events each having 4.9 mm (depth from source area) of runoff, a field-to-buffer strip ratio of 30:1, total loss of atrazine and metolachlor at 5%, loss of pendimethalin at 1%, and a 70% infiltration rate. All pesticide loss was assumed to occur during the first two weeks. This resulted in the following regime: week 1- 840 ml water added with 1.5 mg/L atrazine and metolachlor, 0.30 mg/L pendimethalin; week 2- 840 ml water added with 0.75 mg/L atrazine and metolachlor, 0.15 mg/L pendimethalin; weeks 3 and 4 - 840 ml water added (no herbicide).

Artificial runoff was added to each column drop-wise through a separatory funnel suspended 10 cm above the column. The rate for each event was set initially at 7 ml/min. If water backed up in the column, the rate was slowed to a point that maintained less than 2 cm of standing water. The time necessary for all of the artificial runoff to enter the soil column was recorded (2 hours minimum due to delivery technique). Leachate was collected from the bottom of each column for the following 18 hours, providing adequate time for the column to release excess water.

*Analysis of Leachate and Soil Samples*

Leachate samples were extracted by solid-phase extraction followed by analysis using gas chromatography equipped as previously described [15]. Solid-phase extraction tubes (SPE, Envi-18, Supelco, Bellefonte, PA, USA) were conditioned with ethyl acetate (3 ml),

methanol (3 ml), and water (5 ml). The water sample (200 ml) was passed through the tubes at a rate of 10 ml / min. Tubes were allowed to air-dry for 4 minutes followed by addition of three 4-ml aliquots of ethyl acetate in order to elute herbicides off the column. The ethyl acetate aliquots were pooled and evaporated to 5 ml under a stream of nitrogen. Analysis was performed using a Varian 3400 Gas Chromatograph equipped with a thermionic specific detector (GC-TSD; Walnut Creek, CA). Samples were analyzed on two different phase columns in order to decrease matrix interferences (DB5, DB1701; 15m 0.25 inner diameter, 100μm film thickness, J&W Scientific, Folsom, CA, USA). Extraction efficiency for this method was 96 % (standard deviation 6) for atrazine, 87 % (SD 11) for deethylatrazine, 75 % (SD 14) for deisopropylatrazine, 94 % (SD 5) for metolachlor, and 80 % (SD 3) for pendimethalin.

One week following the final addition of artificial runoff, vegetation was removed from the volumes and measured for dry mass. The soil from each column was divided into two sections (top 14 cm and bottom 14 cm), large root material and rocks were removed, and the soil was chopped with metal spatulas until all clumps were smaller than 5 mm. The soil was then immediately extracted and used in soil activity assays.

Each section, top and bottom, of each column was analyzed to determine the amount of herbicide and atrazine degradation products remaining in the column. Twenty grams (dry wt.) of each soil sample was extracted three times with 60-ml ethyl acetate. The extract was filtered and concentrated to 10 ml using cyclic nitrogen flow while the extract was heated to 55°C. Samples were analyzed similarly as described for water samples. Extraction efficiency for this method was 91% (SD 8) for atrazine, 85% (SD 10) for deethylatrazine, 87% (SD 9) for deisopropylatrazine, 95% (SD 8) for alachlor, 103% (SD 7) for metolachlor, and 95% (SD 11) for pendimethalin.

*Soil activity measurements*

General microbial activity was estimated by measurement of dimethylsulfoxide (DMSO) reduction to dimethlysulfide (DMS) similarly to previously reported methods (16). The method is rapid and inexpensive. Ninety-six percent of bacterial and fungal strains tested were able to carry out this reaction, yet few strains were able to increase microbial numbers solely on DMSO [16]. These characteristics make it a good general marker for

microbial activity. Soil (0.5 g) from the top section of each column was placed into a 13-mm diameter glass test tube. After addition of 0.125 ml of 6.6 % DMSO solution, each vial was capped using a rubber septum. The tubes were incubated for 2 hours at 30° C. Air from each vial (0.5 ml) was injected, using a gas-tight syringe, into a gas chromatograph (Varian 3700) equipped with a 15-m capillary column (DB5, 15 m, 0.25 mm inner diameter, 100 μm film thickness, J&W Scientific) and a flame-ionization detector (GC-FID). The inlet was 30° C, detector 300° C, and the oven was isothermal at 30° C. Samples were compared using external calibration to standards prepared by measuring 10 μl of cold DMS (4° C, liquid) and adding to a test tube capped with a septum. After warming to 25° C, the DMS was entirely gaseous, and volumes of air were used to dilute the stock into a serial dilution which was used to prepare an appropriate calibration curve.

Soil from the top section of each column was also assayed to directly determine the degradative potential of the soil in regards to atrazine and metolachlor. Forty grams of each soil sample was added to each of two 250-ml jars. The first set of jars was fortified with $^{14}$C-atrazine (U-ring) and the second set with $^{14}$C-metolachlor (U-ring). Both radiolabelled pesticides were provided in kind by Novartis (currently Syngenta; Greensboro, NC, USA) and purified in our laboratory to a radiopurity of greater than 96% as determined by thin-layer chromatography (9:1 hexane: acetone mobile phase). Each radiolabelled pesticide was mixed with unlabelled pesticide to obtain a spiking solution that would deliver 100,000 dpm of radioactivity and 0.20 mg of pesticide (5 mg/kg) in 0.50 ml of acetone. Samples were shaken vigorously to mix the pesticides, and then allowed to set for 6 hours to allow volatilization of the acetone carrier. Moisture was added to each soil sample in order to reach the estimated field capacity (21 %).

Samples were incubated in the dark, at 25° C, for 24 days. Vials containing 15 ml of 1 N KOH and a polyurethane foam cylinder (1-cm diameter, 3-cm height) were secured to a phenylbutyl stopper. Each vial was sealed with a stopper, allowing the KOH and foam traps to be suspended above the soil. KOH "traps" $^{14}$C-$CO_2$, while foam "traps" $^{14}$C-organic volatiles. Every 48 hours, the seal was broken to allow fresh air into the jar and to adjust moisture if needed. KOH and foam traps were replaced at 8-day intervals. Aliquots of the KOH trap were added directly to scintillation cocktail and immediately counted using liquid

scintillation counting (LSC; Beckman, Fullerton CA, USA). Foam traps were soaked in 1:1 hexane:acetone for 24 hours, and an aliquot of the solvent was measured using LSC.

After 24 days, the soil in metolachlor treatments was extracted three times with 100 ml of methanol directly in the jar. Samples fortified with $^{14}$C-atrazine samples were not extracted since extensive mineralization occurred during the 24-day incubation period, providing a measurement of activity in regards to atrazine degradation. The jars were shaken vigorously for 20 minutes during each extraction. The solvent extract was filtered, combined, and evaporated to a 5-ml final volume under a stream of nitrogen applied in a manner to create cyclic movement of solvent while the extract was heated to 55° C. Total extractable radioactivity was determined by directly measuring an aliquot of each extract. Each extract was also passed through a reverse-phase high pressure liquid chromatography (HPLC) system to obtain fractions corresponding to parent metolachlor, polar metabolites (early eluting compounds), and two metolachlor degradates, 4-(2-ethyl-6-methylphenyl)-5-methyl-3-morphilinone and N-(2-ethyl-6methylpnenyl)-2-hydroxy-N-(2-methylethyl)-acetamide. The HPLC (Hewlett Packard 1100 series; Palo Alto, CA, USA) was equipped with a $C_{18}$ column (4.6 mm × 25 cm Adsorbosphere, Altech, Deerfield, IL, USA). The mobile phase started as 100% deionized water and followed a gradient to 100% acetonitrile at 10 minutes. After extraction, soil was allowed to air dry, followed by combustion of a 0.5 g-soil aliquot using a Packard Sample Oxidizer (Packard Instrument, Meriden, CT, USA). The resulting carbon dioxide was trapped in Carbosorb and Permofluor (Packard Instrument). Radioactivity was determined by liquid scintillation counting.

*Statistical Analysis*

Data collected in the study were analyzed using a completely randomized design analysis of variance (ANOVA). Comparison among treatments was conducted using Fisher's PLSD ($p<0.05$). Linear regression analysis (Simple Regression) was conducted comparing the dry biomass to all other endpoints where significant differences were found among treatments. All computations were made using Statview for Windows [17]. Standard errors (SE), or standard deviation (SD) where appropriate, are reported in parentheses following all mean values.

## Results

Each column sustained plant growth, both initially and after the grasses recovered from a period of senescence. The amount of dry, above ground biomass was significantly different among treatments ($F=6.5$, $p=0.002$; Table 2). The warm season grasses including mixed prairie grasses, switchgrass, and big bluestem, had the largest biomasses. The type of vegetation influenced the amount of artificial runoff to leach entirely through the column ($F=8.2$, $p<0.001$). All grass types reduced the volume of collected leachate as compared to unvegetated columns ($p<0.05$, Table 2). Generally, the differences between grass types were not significant except that mixed prairie grasses allowed less leachate through the column than did fescue ($p<0.05$). Although all types of grass increased the average infiltration time as compared to unvegetated columns ($F=3.02$, $p=0.03$), there were no significant differences among grass types (Table 2).

| Vegetation Type | Above Ground Dry Biomass, g | % Runoff Recovered as Leachate | Time Required for Infiltration, hr |
|---|---|---|---|
| **Unvegetated** | 0 (0) | 85 (3)$^{A}$ | 7.5 (1.2)$^{A}$ |
| **Brome** | 7.4 (1.2)$^{A}$ | 60 (4)$^{BC}$ | 3.9 (0.9)$^{B}$ |
| **Big Bluestem** | 9.8 (1.5)$^{AB}$ | 57 (4)$^{BC}$ | 3.0 (0.8)$^{B}$ |
| **Tall Fescue** | 5.8 (0.8)$^{A}$ | 66 (3)$^{B}$ | 2.6 (0.3)$^{B}$ |
| **Switchgrass** | 13.4 (2.0)$^{B}$ | 61 (4)$^{BC}$ | 3.2 (0.7)$^{B}$ |
| **Mixed Prairie Grass** | 13.8 (1.3)$^{B}$ | 51 (5)$^{C}$ | 4.5 (1.7)$^{B}$ |

Table 2. Measurements recorded from the soil columns included dry biomass of plant material, the percentage of applied artificial runoff recovered as leachate, and the time required for runoff to completely enter the column. Averages are presented with standard errors in parenthesis. Treatments that are not marked with the same letter were significantly different ($p<0.05$).

*Pesticide mobility*

Pendimethalin was not detected in leachate samples (<0.18% of applied). There were no significant differences found among grass types for either the total amount of

pendimethalin found or for the ratio found in the top section versus the bottom section. The average pendimethalin recovery inclusive of all treatments was 67% (SE 3.8%). On average greater than 83% (SE 0.98) of the pendimethalin recovered was from the top section.

Atrazine was found throughout the soil columns and in leachate for every column. The total amount of atrazine recovered from the leachate during the entire study was not significantly different among treatments (Figure 1). However, during the final two weeks, significantly less atrazine was recovered from leachate in mixed prairie grasses, switch grass, and big bluestem treatments as compared to the unvegetated treatment ($F=3.5$, $p=0.02$; Figure 1). There were no significant differences between the total amounts of atrazine recovered from the leachate and soil column combined. The average percentage recovered ranged from 48 % (SE 6%) for unvegetated columns to 56 % (SE 6%) for columns treated with brome. There were also no significant differences among treatments regarding the distribution of atrazine among the column sections and the leachate. Overall, 51 % (SE 2%) of the recovered atrazine was found in leachate, 15 % (SE 1%) was found in the bottom section, and 34% (SE 2%) was found in the top section of the columns.

One atrazine metabolite, deethylatrazine (DEA), was detected in every leachate sample during the last three leaching events. It was not found in the initial event. The amount of recovered DEA was significantly different among treatments ($F=5.0$, $p=0.003$). Significantly more DEA (0.55 %, SE 0.06) was recovered from unvegetated columns as compared to all vegetated columns. No differences were found among columns vegetated with grass species where the mean recoveries of DEA ranged from 0.26 % of added (SE 0.03) for brome to 0.35 % (SE 0.07) for mixed prairie grasses. Another atrazine metabolite, deisopropylatrazine (DIA), was only detected above detection limits in 6 samples ($n=120$). Overall, total DIA recovery from leachate was below 0.18 % of added atrazine for all treatments. Neither compound was found in soil above the detection limit of 8 % of added atrazine.

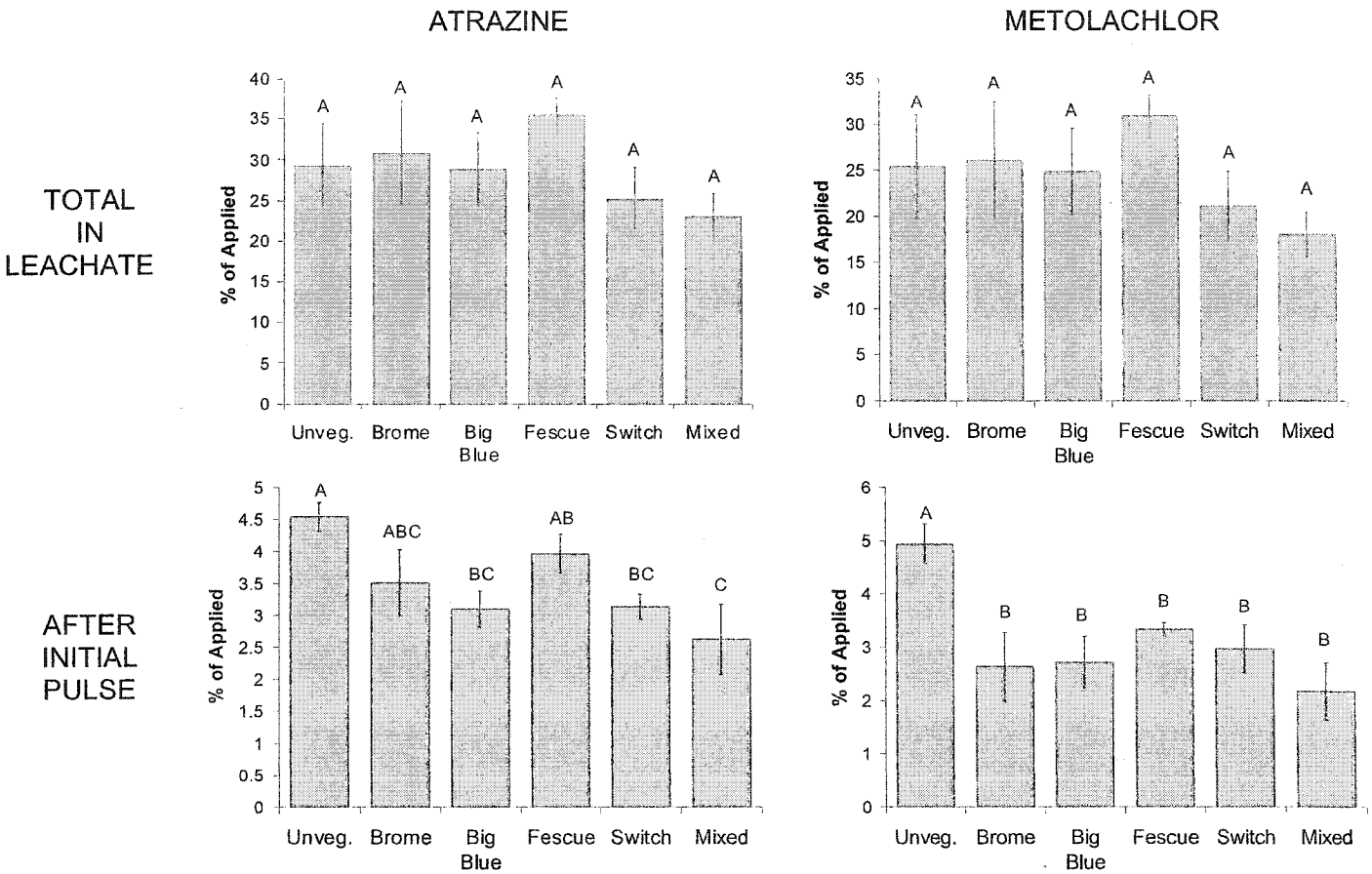


Figure 1. The total amount of atrazine and metolachlor recovered from leachate throughout the study was not significantly different among treatments. However, the amount that was recovered for leachate during the final two clean water runoff events – after the initial two additions of herbicide – was significantly reduced by vegetation ($p<0.05$). Treatments that are not marked with the same letter were significantly different ($p<0.05$).

Metolachlor was also found throughout the soil columns and in leachate for every column. As with atrazine, the total amount of metolachlor recovered from leachate was not significantly different among treatments; however, the amount of metolachlor recovered from leachate during the final two weeks was significant different ($F=4.33$, $p=0.006$; Figure 1). All grass species reduced the amount of metolachlor in leachate during this period as compared to controls; however, no differences occurred among grass types. There were no significant differences among treatments regarding the total amount of metolachlor recovered from leachate and soil columns combined, or the ratio of that recovered in each section of the column and the leachate. The total amount recovered ranged from 50% (SE 5%) in mixed prairie grass columns to 66% (SE 4%) in the fescue columns. Overall, 38% (SE 3%) of the recovered metolachlor was found in leachate, 49% (SE 3%) was found in the top section, and 13% (SE 1%) was found in the bottom section of the columns.

*Rhizosphere activity*

Atrazine mineralization occurred rapidly in the soil collected from the columns, resulting in over 40% mineralization in all treatments within 24 days. After 24 days, there were no significant differences among treatments; however, after 8 days, there were significant differences among treatments ($F=3.38$, $p=0.02$). As shown in Figure 2, mixed prairie grasses and brome had the highest amount of atrazine mineralization, while unvegetated and fescue treatments had the lowest amount. Since, the amount of atrazine mineralization was high after 24 days, and there were no longer differences between treatments, no further analysis of these samples was conducted.

Metolachlor degradation was much slower than that of atrazine in the collected soil. Negligible activity (less than 0.5 % of added radioactivity) was found in volatile and $CO_2$ traps. All of the radioactivity added was recovered from the soil either by solvent extraction or by combustion of the extracted soil. There were no significant differences in the mass balance among treatments, with an overall average of 102% (SE 2, n=30). Greater than 90% of the extractable radioactivity was determined to be metolachlor parent, the other 10% was primarily unidentified metabolites eluting prior to the parent compound and standards of two chlorinated metabolites. There were no significant differences among treatments regarding

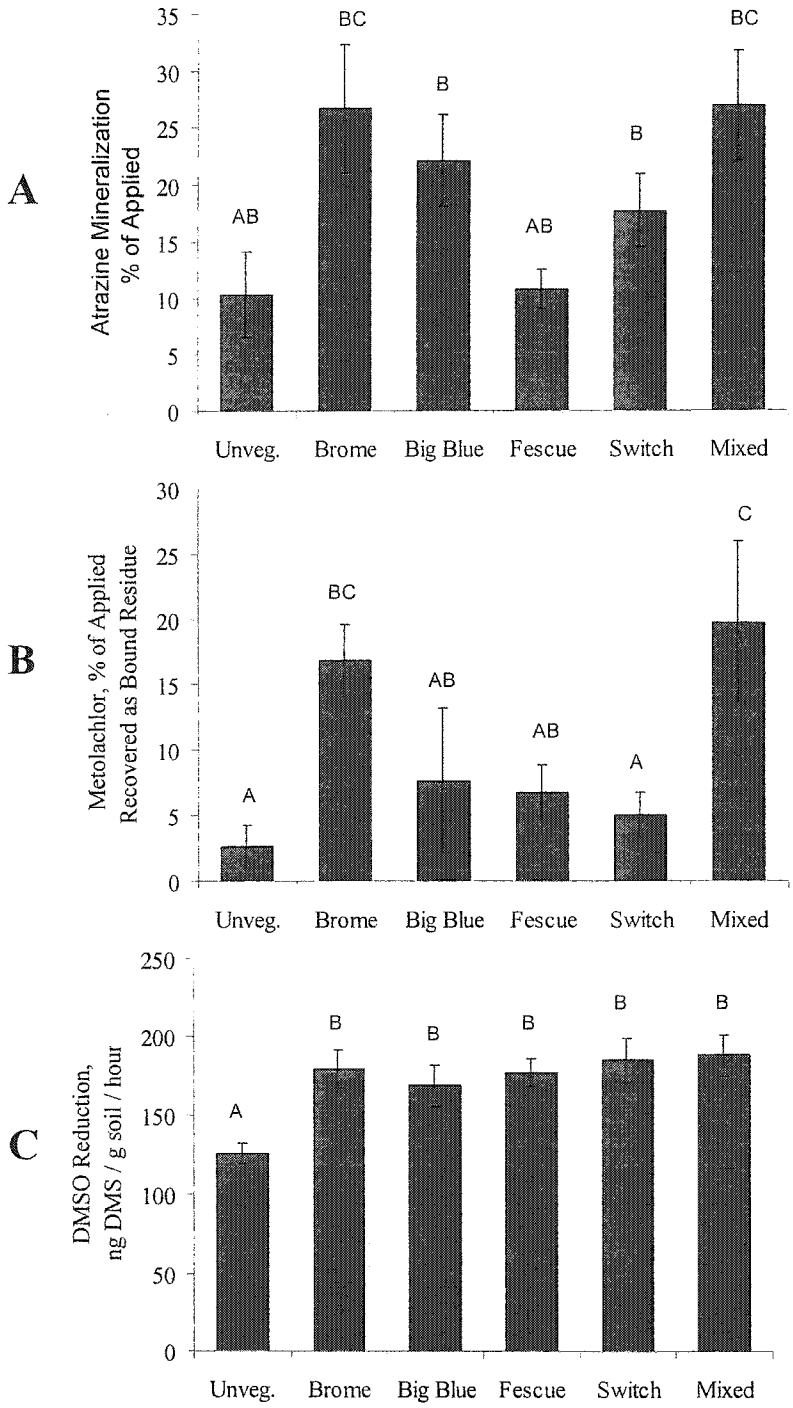


Figure 2. Chemical transformation ability of the soil from each column was measured by atrazine mineralization, amount of metolachlor bound residue formed, and the rate of DMSO (dimethyl sulfoxide) reduction. Treatments that are not marked with the same letter were significantly different ($p<0.05$).

the amount of metabolites present in the extracts. However, the percentage of radioactivity extractable and metolachlor extractable were significantly different ($F=3.27$, $p=0.02$; Figure 2). The amount not extractable was found in the bound residues of the soil as determined by soil combustion.

Reduction of DMSO to DMS was found to be different in unvegetated compared to vegetated treatments ($F=3.9$, $p=0.01$; Figure 2). However, there were no significant differences among vegetated treatments.

*Endpoint regression with biomass*

All endpoints of the study that showed significant differences among treatments were tested for correlation with biomass using linear regression. Table 3 shows the results for these analyses. Although four of the six endpoints had a significant response to increases in biomass, only the volume of leachate recovered had a correlation coefficient above 0.50.

## Discussion

Infiltration has been reported to be one of the most important processes in the removal of pesticides from surface runoff by VFS [6, 7, 14]. In this study, the presence of grasses greatly increased the infiltration rate of water into the soil columns as compared to the unvegetated columns (Table 1). Although mean infiltration times differed among vegetation types by 70%, the values were not statistically significant. The high variability present among the intact soil columns was greater than the variability caused by difference among grasses. Further study with either more controlled soil columns or greater replication would be necessary to fully differentiate between grass types. A variety of factors may influence the infiltration rate of VFS including slope and soil type. Interaction between grass types and these other factors may occur. Time necessary for infiltration of a known volume to occur as measured in this study provides a base measure of infiltration performance; however, further studies using tilted-beds of soil would better reflect the complexity of infiltration into VFS while allowing for a controlled environment.

Substantial biomass was obtained on the soil columns. Tall fescue yielded 7.1 Mg/ha (5.8 g / column) and mixed prairie grasses treatments yielded 17 Mg/ha (13.8 g / column) of dry biomass. As a comparison, switchgrass grown for harvestable biomass on two Oklahoma

| Parameter Correlated with Biomass | Analysis with all columns n=30 | | Analysis using treatment means, n=6 | |
|---|---|---|---|---|
| | ANOVA Result | Correlation Coefficient | ANOVA Result | Correlation Coefficient |
| **Leachate volume** | F=53, $p<0.001$ | $r^2=0.66$ | F=16, $p=0.02$ | $r^2=0.80$ |
| **Infiltration Time** | F=1.7, $p=0.19$ | $r^2=0.06$ | F=2.4, $p=0.20$ | $r^2=0.37$ |
| **Atrazine Leaching, Final 2 Weeks** | F=15, $p=0.006$ | $r^2=0.35$ | F=1.7, $p=0.26$ | $r^2=0.31$ |
| **Metolachlor Leaching, Final 2 Weeks** | F=20, $p<0.001$ | $r^2=0.41$ | F=3.3, $p=0.14$ | $r^2=0.46$ |
| **Atrazine Mineralization, 8 day** | F=7.7, $p=0.01$ | $r^2=0.22$ | F=3.1, $p=0.15$ | $r^2=0.44$ |
| **Metolachlor Formation of Bound Residues, 24 day** | F=2.8, $p=0.11$ | $r^2=0.09$ | F=1.2, $p=0.32$ | $r^2=0.24$ |

Table 3. Results of regression analysis correlating the dry above-ground biomass from each column to other endpoints measured. Each endpoint listed showed statistically significant differences among treatments.

(USA) sites, had average yields ranging from 6.7-19 Mg/ha [18]. The greenhouse conditions and long growing season allowed for better growth than would be expected in the first year of these perennial grasses. Warm season grasses (big bluestem, switchgrass, and yellow indiangrass) showed better growth in the greenhouse conditions than did the cool-season grasses (tall fescue and smooth brome). In all vegetated columns, roots extended completely through the column and out of the bottom end.

Atrazine and metolachlor exhibited a high degree of mobility in this study. As indicated in Figure 1, an average of 25% of the added atrazine and 24% of metolachlor was recovered in leachate. Over 20% of the added amount was recovered during the first two leaching events for each pesticide, the time period that the pesticides were added to the

column. These high initial recoveries, followed by much smaller recoveries, likely indicate that much of the pesticide movement was due to transport in macropores within the soil column. Several intact-column mobility studies have reported a much lower degree of mobility for $^{14}$C-atrazine and $^{14}$C-metolachlor [19, 20]. In the atrazine study, less than 2% of the applied radioactivity was found in leachate, while in the metolachlor study less than 4% of the applied radioactivity was recovered in leachate, with only 1.09% recovered as metolachlor. Both of these studies lasted 12 weeks. However, these studies differed from the current study in several ways in regard to method design. Both studies were conducted based on field application and field leaching. Therefore, the pesticides were incorporated into the soil and allowed to incubate for three weeks before a leaching event. In addition, the columns were longer, a smaller volume of water was applied (to mimic rainfall only, instead of a VFS which receives rain and runoff), and the columns were saturated from below before leaching began. The higher degree of mobility found in this study as compared to traditional studies may warrant concern regarding the potential impact of runoff contaminating ground water. Pesticide leaching through VFS and into groundwater may not be adequately predicted using models designed to describe leaching of pesticides applied to fields.

Limited studies are available that have investigated the mobility of pesticides applied to soil columns in artificial runoff. One of these studies was conducted on tilted soil beds, constructed by packing soil into an aluminum frame, and using switchgrass to create VFS [7]. In this study, artificial runoff, fortified with atrazine and metolachlor, was applied to the bed and allowed to run over the surface. Of the amount that infiltrated, 10% of the atrazine was recovered from leachate in vegetated treatments, while 4% was recovered from unvegetated treatments. Similarly, 6% of metolachlor was recovered from leachate in vegetated treatments, while 3% was recovered from unvegetated treatments. Although the mobility in this study is lower than in the current study, the artificially prepared tilt-beds probably had little macropore movement, especially in the unvegetated treatments.

The lack of pendimethalin mobility found in this study followed similar trends that were previously reported. Pendimethalin has been reported to have limited soil mobility after agricultural applications [2]. Although some reports have suggested that pendimethalin may leach in small quantities through macropores [21], it was not recovered in leachate during this study, even though the other herbicides leached at relatively high levels.

Many soil column studies use a standard procedure of saturating the column from below to fully saturate the soil and then allow drainage for 16 hours prior to the leaching study. This technique is used to remove air bubbles from the column and provide a uniform starting condition [19, 20]. However, this method has been reported to collapse artificial macropores [21]. Instead of this protocol, the current study followed a microcosm type of approach. Prior to the addition of artificial runoff, columns were saturated from above and allowed to dry for two days prior to leaching. The philosophy behind this approach is that saturation from the top likely protects soil column changes due to vegetation such as development of macropores to allow preferential flow of water and evapotranspiration-driven drying of the column and allows these events to have an impact on the soil column hydrology as would occur in the environment. Additionally, the current method design consisted of adding artificial runoff to the columns over a one month period. During this time, the initial advantage of a consistent moisture balance would quickly diminish since the moisture balance would be constantly changing in the greenhouse conditions needed to keep the plants healthy. The disadvantage of the microcosm method design is that care must be used when comparing the results of this study to other column mobility studies. However, little direct flow through macropores in the column was occurring since pendimethalin was not recovered in any leachate samples.

Even though the total amounts of metolachlor and atrazine that leached through the column were not significantly different among treatments, the differences found in the last two weeks indicate that reduced pesticide leaching may occur in vegetated columns. In the first two weeks, the artificial runoff was fortified with atrazine and metolachlor. The amount that did not come through during this initial flush, moved out of the column at a lower rate. The columns used in this study were only 30 cm, only a fraction of the distance pesticides usually have to travel to reach groundwater. Longer columns would likely result in greater initial retention and thus the vegetation may, ultimately, have a greater effect. In addition, it is positive that vegetation increased infiltration rates, yet held mobility steady or at a decreased rate.

Reduction of the amount of pesticide leaching may be partially due to creation of greater numbers of macropores – creating preferential flow routes. Several investigators have reported that preferential flow may increase the initial amount of low and moderately

sorbing pesticides (such as metolachlor and atrazine) that will leach. However, subsequent leaching events will result in less movement due to the direction of flow through preferential channels and away from soil areas still holding pesticides [21, 22]. Soil organisms that create macropores, such as earthworms, may also decrease pesticide transport by moving pesticide residues away from the surface and by generating organic matter that may increase binding sites [22]. Vegetation may have similar, albeit slower mechanisms that result in increased organic material in the soil that helps sorb pesticides more tightly. A second mechanism involved in reduced mobility of pesticides is simply changes in the water movement within the soil column. In this study, vegetative treatments dried the soil columns (as demonstrated by the reduced recovery of leachate volume in Table 2). The reduced downward movement of water in the column may have simply been enough to reduce the downward pesticide movement due to upward movement of water from the transpiration stream.

In order for VFS to be successful, pesticides must be trapped within the soil or plant material, and then they must undergo biotransformation (usually detoxified, if not mineralized). In this study, soil activity measurements indicated that vegetation in general increased atrazine mineralization, metolachlor bound residue formation and general microbial activity as determined by DMSO reduction (Figure 2). Although some grass species performed better than others in increasing atrazine degradation, they were basically equivalent in total microbial activity and in metolachlor degradation. Another recent study reported similar findings that vegetation increased microbial activity and pesticide degradation rates. When soil from a VFS was compared to barren soil nearby, the VFS soil had higher microbial activity as measured by a variety of endpoints, higher adsorption rates for metolachlor, and higher rates of metolachlor degradation [23]. Other studies have demonstrated that the addition of plants helps increase dissipation of pesticide from contaminated soil [24, 25].

Atrazine persistence in soil varies depending on concentration, soil type, and previous exposure history [26, 27]. The amount of mineralization also is variable. One study reported only a few percentage of applied atrazine was mineralized within 28 days [27], while another study reported up to 70% mineralization [26]. The past history of the field site which includes multiple applications of atrazine, may explain why the rate was so high. In several

studies, the amount of mineralization stabilized at 40-60% of applied at the time point where soil residues diminished. Due to this, no further effort was invested in analyzing the soils from the atrazine study. Metolachlor degradation rates and pathways (mostly bound residues) were similar to what have been reported previously [23].

When comparing among plants of different species, differential growth rates – resulting in different biomasses – may be a primary factor behind differences. However, in this study, regression analysis between biomass and the endpoints measured yielded little evidence that biomass was the primary factor. Only the volume of leachate recovered had a correlation coefficient of greater than 0.50 (Table 3). The low correlation coefficients and low probability of a correlation even existing (based on non-significant ANOVA results) indicates that the species change the physical/chemical environment of the soil column either to a different extent, or in different ways, that are unrelated to simple differences in biomass. Several issues of method and data interpetation invariably arise when conducting microcosm type studies. For instance, did the root systems developed differently in columns versus continuous soil? Are evapotranspiration rates and therefore moisture cycles similar to what would occur naturally? Although this type of study cannot answer all of these questions, the small, inexpensive design allows for measuring the flow of contaminants in soil and comparing multiple vegetation types, a factor that is often neglected in fate studies involving soil and VFS. Additionally, this method design treats the soil column as a system and provides knowledge about the fate of the contaminant within a system instead of simple soil degradation studies or leaching studies in a saturated column per more commonly used designs. Data provided from this study should provide insight into future field studies and pesticide fate studies that work with a few treatments in a more rigorous manor.

Implementation of filter strips can help reduce sediment and nutrient loading of streams, prevent pesticide runoff, and provide habitat for wildlife. When grass species are chosen, a variety of variables may be considered. To our knowledge, this is the first work to provide input regarding how grass species may influence the fate and flow of pesticides within filter strips. The results of this study suggest that several options may be equally as good as far as grass species selection. In general, few differences were found among species. Two commonly used species, smooth brome and switchgrass, performed well for almost all endpoints. Fescue allowed for rapid infiltration; however, for most other endpoints, fescue

performed similarly to unvegetated treatments. In most of the endpoints evaluated in this study, the mixture of prairie grasses was the best or among the best. This information may prove valuable to those implementing multi-use filter strips in corn/soybean agricultural regions of the USA, since this native mixture would also provide habitat for wildlife.

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# CHAPTER 4. FATE OF [$^{14}$C] – PENDIMETHALIN IN UNVEGETATED AND PRAIRIE GRASS-VEGETATED SOIL

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**A paper submitted to *Environmental Toxicology and Chemistry***

**Abstract**

Due to heavy usage and high persistence in soil, the herbicide pendimethalin is of environmental concern, especially at agrochemical dealerships where heavy contamination may occur due to incidental spillage. Recently, prairie grasses have been suggested as a remediation tool for reducing the impact of point-source (phytoremediation) and nonpoint-source (filter strips) contamination by pesticides. The objective of this study was to measure the fate of $^{14}$C-pendimethalin in unvegetated and prairie grass-vegetated systems. Vegetated and unvegetated soil systems were placed in enclosed, flow-through chambers allowing measurement of soil concentrations, uptake into the grass, mineralization, and volatilization. Pendimethalin dissipation occurred in soil from both unvegetated (44 % of initial) and vegetated chambers (37 % of initial), with significantly more loss from soil in vegetated chambers. The amount of uncharacterized, methanol-extractable metabolites significantly increased in vegetated chambers (6.7 % unvegetated, 7.8 % vegetated). The amount of mineralization significantly decreased in vegetated columns from 9.0% to 7.2%, as did the volatilization (0.48% to 0.29%). Although 3.1% of the radioactivity in vegetated chambers was recovered from plant tissue, 80-90% was bound, and only 1% of the radioactivity in the stem and leaves and 10% in the roots were attributable to parent pendimethalin. The differences in pendimethalin dissipation from soil indicate that plants do increase pendimethalin dissipation, while not burdening the surrounding air and plant tissue with high levels.

**Key words**: pendimethalin, phytoremediation, prairie grass, vegetative filter strips, plant uptake

## Introduction

Pendimethalin, N-(1-ethylpropyl)-3,4-dimethyl-2,6-dimethyl-2,6-dinitrobenzeneamine – a dinitroanaline herbicide, is used throughout the world in numerous crops including cotton, soybeans, corn, sorghum, potatoes, sugarcane, tobacco, turf, winter cereals, and vegetables. Pendimethalin provides control of annual grasses and certain broadleaf weeds by inhibiting seedling root growth through disruption of mitotic cell division [1]. Throughout the last 30 years, large amounts of pendimethalin have been applied to soil. For example, in 1996, 6 million kilograms of pendimethalin were applied to soybean fields in the United States [2].

Pendimethalin has low mobility in soil [3] and is generally considered to be a very limited threat to ground and surface water. However, it can be quite persistent after incorporation into soil with field dissipation half-lives ranging from 47 days [4] to 407 days [5] depending on temperature and soil characteristics. In one study, greater than 50% of previously aged pendimethalin residues remained in the soil after 1000 days [6]. Due to this long environmental persistence, pendimethalin is one of the few current-use pesticides to be added to the United States Environmental Protection Agency's list of Persistent Bioaccumulatory and Toxic compounds under the Toxic Release Inventory program [7].

Intensive usage of pendimethalin, and other herbicides, can result in environmental contamination either by intentional application, followed by environmental movement through surface runoff, leaching into groundwater, or volatilization, or by incidental spillage during manufacturing, mixing, or loading [8]. Studies have reported environmental contamination of pendimethalin in groundwater near agrochemical dealerships [8] and in surface water [9]. Another study, investigating pesticide contamination in soil at agrochemical dealerships, revealed pendimethalin concentrations above 200 mg/kg [10].

In the last few years, studies have shown that prairie grass can be a useful tool for reduction of pesticide contamination. Several investigators have shown reduced pesticide runoff from fields when switchgrass and other grasses are used as filter strips [11, 12]. Additional studies have indicated that phytoremediation, using prairie grasses as the remediation agent, may be a viable option for mitigation of pesticides, including pendimethalin, at agrochemical dealerships [10, 13].

Although success in early studies has led to rapid implementation of filter strips [11] and phytoremediation into field projects [14], questions often remain unanswered regarding

the usefulness of the techniques. For example, little research has been conducted on the mechanisms of herbicide dissipation from contaminated soil vegetated with grasses, including the role of uptake into the plant, degradation in the root zone, and volatilization from the plant or soil. Better understanding of these processes could greatly enhance our ability to use grasses as mitigating agents for pesticide contamination and aid in decision-making about the usefulness of the techniques.

The objective of this study was to track the fate of pendimethalin in soil systems vegetated with prairie grass and in soil systems left unvegetated. To accomplish this, $^{14}C$-pendimethalin was added to soil in a contained system. After 108 days, quantitative measurement of the transformation of the herbicides in soil and the amounts of herbicide and metabolites released to other compartments within the system (plant, air) were performed.

## Methods

*Construction of Chambers and Traps*

Eight test chambers were constructed using a large PVC (polyvinyl chloride) cleanout fitting designed to cap 10-cm inner diameter (ID) PVC pipe. Acrylic pipe (11-cm ID, 60-cm length, Aquatic Ecosystems, Apopka, FL, USA) was sealed into the cleanout using PVC cement (Oatley, Cleveland, OH, USA). The top was enclosed with a flat sheet of acrylic using acrylic cement (WELD-ON 16; IDS, Gardena, CA, USA). Acylic tubing (5-mm ID) was inserted into a hole drilled in the PVC base and sealed with acrylic cement. This tube served as an air inlet. A second piece of acrylic tubing (5-mm ID) was placed in the flat acrylic top for use as an air outlet. In order to allow easy access to the chamber, a 15-mm hole was placed in the side of the chamber 8 cm above the PVC fitting. The hole was sealed with a butyl rubber stopper when not in use. Air flowing into the chambers consisted of ambient air from the greenhouse pumped through a PVC manifold and into the inlet. High flow was used (7 ml/sec, which provided a complete turnover within the chamber approximately every 13 minutes) in order to remove $^{14}C$-$CO_2$ and volatile organics from the system as quickly as possible. Flow was checked periodically during the test and maintained within 10 % of target value.

Outlet air passed through a series of traps in order to determine mineralization and volatile organics (including volatilized pendimethalin). Immediately attached to the outlet

was a volatile organic trap consisting of a polyethylene drying tube (1.5-cm ID; Bel-art Product, Pequannock, NJ, USA) containing a polyurethane foam cylinder (1.7-cm diameter, 6-cm length; Applied Products, South San Francisco, CA, USA). The foam was 2 mm larger than the holder, requiring slight compression to insert the foam and sealing the foam against the holder. Nalgene® tubing connected the volatile organic trap to the $CO_2$ traps that consisted of two 40-ml glass vials capped with rubber-Teflon septa. The connecting tubing was pulled through small holes drilled in the septa and were capped with 18 gauge disposable needles 3.8 cm long (Becton Dickinson, Franklin Lakes, NJ, USA). The end of each needle was placed near the bottom of the vial. Both vials were filled with 20 ml of 2N KOH. The outlet of the traps consisted of disposable needles piercing the septa. The needles were attached to tubing that led to the next trap. A final trap, built identical to the $CO_2$ traps, except filled with ethylene glycol, was added to the system. This final trap served as a second trap for volatile organics. It also provided uniform resistance to the air-flow, which helped to regulate flow consistently among columns.

In order to obtain a semi-quantitative measure of the transfer of radioactivity to plant leaves through volatilization of pendimethalin or metabolites, an internal volatile trap was also used. This trap consisted of a polyurethane foam cylinder (1.7-cm diameter, 3-cm length; Scientific Products) attached to the butyl rubber plug in the side of the chamber. The trap was located about 3 cm directly above the soil during the test.

*Treatment of soil*

Agronomic soil was obtained from a field site near Ames, IA, USA that had not received herbicide treatment for over 10 years (Field 55, ISU Ag Engineering/Agronomy Farm). The soil was sieved (2.8 mm) to remove plant material and rocks. Analysis of the soil indicated a sandy loam texture (60% sand, 22% silt, and 18% clay), 2.7% organic matter, and a pH of 7.0 (Midwest Laboratories, Omaha, Nebraska, USA). Soil was collected as a composite from a field mapped as Nicollett and Webster. The soil was stored at 4°C in polyethylene bags prior to use (less than 7 days).

Pendimethalin radiolabelled with $^{14}C$ in the aromatic ring was received as a gift from American Cyanamid (currently BASF). The radiopurity of the stock was determined to be greater than 96% by thin layer chromatography using the methods later described for

examination of soil extracts. Radioloabelled pendimethalin was diluted in acetone along with analytical grade pendimethalin (98 % purity, Chemservice, West Chester, PA, USA) to obtain a spiking solution of 3 mg/ml pendimethalin and 1.4 μCi/ml. Ten ml of stock was added to 1500 g of soil in 4-L amber jars to obtain a soil concentration of 20 mg/kg. The jars were rotated for 4 hours on their side at a rate of 20 rotations per minute with frequent venting. Four batches were made. The batches of soil were then adjusted to 17% moisture (slightly below estimated field capacity), sealed, and placed in an incubator at 16° C, in the dark for 160 days. Moisture was adjusted and the jars were vented weekly. Following the aging, soil was extracted and analyzed as later described for soil samples evaluated post-test. During the aging process some degradation occurred. The distribution of radioactivity among parent, metabolites, and bound residue in soil is shown in Table 1. Greater than 96 % of applied radioactivity was still in the soil at the end of the aging period.

Plugs of grass were prepared in cone containers (1.5 cm x 4 cm). Three weeks prior to starting the study, 20 g of agronomic soil from the same source as previously described was added to each cone. Fifteen seeds were added to each cone, except for cones intended for unvegetated columns, which were left unplanted. Cones were planted with either big bluestem (*Andropogon gerardii*), yellow indiangrass (*Sorghastrum nutans*), or switchgrass (*Panicum vergatum*) using seed purchased from United Seed Company (Omaha, NE, USA). Each plug was fertilized with 5 ml of an aqueous solution containing 0.005 % N, 0.0075 % P, and 0.005 % K (plus trace nutrients, Schultz Plant Food, St. Louis, MO, USA) at planting and after two weeks.

*Addition of plants to pendimethalin-treated soil*

Following the 160-day aging period, 600 g of fortified soil from each soil batch was added to each of two 700-ml amber jars (5.5 μCi total). For each pair of jars, one was randomly assigned as a vegetated and the other as an unvegetated treatment. For the vegetated treatment, one plug of each grass type was added (3 plugs) and for the unvegetated treatment, 3 unvegetated soil plugs were added. The soil was gently packed into the jar to obtain a soil density of 1.1 g/cc. To encourage plant growth, $NH_4NO_3$ was added to soil in an aqueous solution (equivalent to 24 kg/ha). The jars were then placed into the appropriate chamber through the cleanout fitting at the bottom of the chamber. To prevent leakage and

seizing in the threaded fitting, Non-hardening Pipe Joint Compound was applied to the threads (consists of Teflon, $CaCO_3$, oil, titanium dioxide; Oatley, Cleveland, OH, USA).

*Monitoring of traps*

Every 3 days, both KOH traps were removed and replaced with fresh aliquots of KOH. Ethylene glycol traps were replaced every 6 days. The volume of each trap was recorded, and 1 ml of the solution was added to 9 ml of scintillation cocktail (Optima Gold, Fisher Scientific, Pittsburgh, PA, USA). Radioactivity was determined by liquid scintillation counting (LSC). To insure that the KOH traps were working efficiently, an aliquot from the each trap was mixed with a $BaCl_2$ solution at a molar ratio of 2:1 Ba:K. This resulted in precipitation of insoluble $BaCO_3$. The solution was then titrated with HCl. All traps tested had greater than 0.2 N KOH remaining. Periodically, radioactivity in the solution was measured. Following precipitation, less than 5% of the radioactivity remained in solution.

Internal and external polyurethane foam traps for collection of volatiles were changed every 36 days. The foam was removed and replaced with fresh foam. The foam from the traps was soaked for 48 hours in 30 ml of 1:1 hexane:acetone. An aliquot of extract was then counted by LSC to determine radioactivity. The efficiency of this extraction technique, tested by adding radiolabelled pendimethalin in an acetone carrier to the polyurethane foam, indicated 101 % recovery (n=4, standard deviation = 2.4). After the final 36-day period, all extracts from foam traps were combined for vegetated chambers and for unvegetated chambers. The combined extracts were concentrated to 5 ml under a stream of nitrogen gas. Aliquots (0.10 ml) of each extract were examined using TLC plates as discussed later for soil extractions. Combining of extracts was performed in order to obtain enough radioactivity that separation of extracts was feasible. Additionally, the polyethylene drying tubes were soaked for 24 hours in ethyl acetate and radioactivity in the ethyl acetate was measured in order to determine if tubes themselves collected radioactivity.

*Soil moisture*

Throughout the study, moisture was added to the soil on a six-day rotation. On day two and four, 10 ml of deionized/reverse osmosis treated water was added to each column (1.5 % mass water / dry mass of soil). On day 6, an estimate of soil moisture was obtained using a

Hold All Moisture Meter (American Tack and Hardware, Monsey, NY, USA). The meter was calibrated against soil from the same source used in the chambers, which had been adjusted to field holding capacity (17 % water/dry soil). Soil in each chamber was probed in two spots to estimate moisture content. The columns were then differentially watered depending on the results. This allowed each system to return to consistent moisture content even though differences existed among columns in regard to water loss.

*Dismantling chambers and processing of soil and plant material*

After 108 days, soil jars were removed from the chambers. Stem and leaves were clipped from the top of the soil. The soil (with the roots in vegetated systems) from each experimental unit was dumped into a glass pan. Roots were separated from the soil using forceps. Although an effort was made to remove all roots from the soil, very small roots were not recovered. Likewise, soil was scraped from the roots; however, the roots were not rinsed so as to avoid losing radioactivity, allowing some soil to cling to the roots. Soil was mixed thoroughly within the glass tray and then shaken vigorously in a glass jar. Leaf and stem samples, as well as root samples, were chopped into sections no longer than 1 cm.

Two 20-g aliquots of soil from each chamber were placed in glass jars along with 80 ml ethyl acetate. The jars were shaken vigorously for 25 minutes and the solvent collected through filter paper. The process was repeated twice and all three extracts pooled and reduced to a 5-ml volume under a stream of nitrogen applied in a cyclic fashion while the extract is heated on a water bath at 50°C. The total radioactivity of the extract was determined using LSC. Extraction efficiency for this technique was 94 % (standard deviation 4.3%) for pendimethalin. Extraction efficiency was determined by adding the spiking solution, as previously described, to soil 6 hours before extraction.

Two 0.5-g portions of the extracted sample were obtained. The first portion was mixed with 0.1 g cellulose and oxidized in a Packard 307 Sample Oxidizer (Packard Instruments, Downers Grove, IL, USA). $CO_2$ was collected in Permafluor and Carbosorb (Packard Instrument) and radioactivity determined by LSC. The second portion was combined with 3 ml of 1:1 methanol:water and placed on a 45° C sonicated water bath for 40 minutes. The solution was then centrifuged to remove suspended soil, and radioactivity was

determined by LSC. Using this technique, radiation in the soil was fractionated into three categories, extractable by ethyl acetate, extractable by methanol/water, and bound residues.

In order to determine the amount of pendimethalin in the soil, in relation to ethyl acetate extractable metabolites, an aliquot of each ethyl acetate extract was chromatographically separated using thin-layer chromatography (TLC). A 9:1 hexane:acetone mobile phase was used on silica gel-coated plates (0.25 μm film thickness, Whatman, Clifton, NJ, USA). The $R_f$ of pendimethalin in this system was 0.75. Plates were separated into four regions: from the origin up to an $R_f$ of 0.20, 0.20 to 0.70, 0.70 to 0.80 (corresponding to pendimethalin) and above 0.80. Each section of each plate was placed in a scintillation vial along with 10 ml of Scintiverse BD cocktail (Fisher, Pittsburgh, PA, USA). After 24 hours, radioactivity in each vial was determined using LSC.

Plant and root samples were ground in ethyl acetate using a mortar and pestle. Four extractions were performed, each with 20-ml of ethyl acetate, grinding for 5 minutes. After grinding, the plant material was nearly colorless and appeared completely macerated. Extracts were evaporated, radioactivity was determined and chromatographically separated as described for soil samples. After extraction, two 0.4 g-aliquots of plant residue were oxidized as previously described. This process used all leaf and stem material.

*Statistical analysis*

Statistical differences between vegetated and unvegetated treatments were conducted using paired T-tests. Each test had 3 degrees of freedom and tested the null hypothesis that the difference between treatments was equal to zero. All means are expressed with the standard error in parenthesis unless otherwise noted. Statistical calculations were performed using Statview [15].

**Results**

All grass plugs survived transplanting and grew in the pendimethalin-fortified soil throughout the course of the study. The mean mass of leaf and stem was 1.50g (SE 0.07), and the mean mass of root recovered was 3.95g (SE 0.37). Based on the experimentally determined percent solid of 13.5%, this is the equivalent to 0.29 Mg/ha above-ground biomass. In the first weeks of the study, the amount of water needed to keep the chambers

moist was not different between unvegetated and vegetated chambers, averaging 6.0 mg water / g dry soil / day (SE 0.2). As the grasses matured, the vegetated chambers required significantly more water (T= 6.54, $p<0.001$) averaging 7.9 mg/g/day (SE 0.3), while the unvegetated chambers maintained the previous watering rate averaging 5.6 mg/g/day (SE 0.1).

Pendimethalin-related radioactivity was found throughout all matrices in each chamber as shown in Table 1. Significantly more total radioactivity was recovered from vegetated columns, 87.3 % (SE 1.8), as compared to unvegetated columns, 83.0% (SE 2.3; T=3.63, p= 0.036). The majority of the radioactivity was recovered from the soil as either extractable or bound residue, followed by the mineralized fraction, plant uptake (in vegetated chambers), and finally volatile organics.

Radioactivity from soil accounted for 91.0 % (SE 0.5) of the total radioactivity recovered from unvegetated chambers and 89.3% (SE 0.4) of the total radioactivity recovered from vegetated chambers. Table 1 shows the distribution within the soil in regards to the total amount of radioactivity found in the chambers. Table 2 illustrates how this distribution changed from the pretest soil in both vegetated and unvegetated systems. In vegetated systems, the total amount of radioactivity that was ethyl acetate-extractable was significantly reduced as compared to unvegetated systems (T=3.47, p=0.041) as was the amount of pendimethalin recoverable (T=4.30, p=0.023). The amount of radioactivity that was not extracted with ethyl acetate, but that was extracted with methanol/water was higher in vegetated soils (T=4.47, p=0.021). The other pools of radioactivity within the soil were not significantly different between vegetated and unvegetated soils.

During the first 36 days of the study, no differences existed between the amount of radioactivity recovered in unvegetated and vegetated $CO_2$ traps (2N KOH). However, as the grasses developed, a steady trend was observed with the vegetated treatments having reduced mineralization resulting in significant differences by the end of the study (T=5.80, p=0.102; Table 1). Recovery of radioactivity as volatile organic compounds followed a similar trend. During the first 36 days, little difference was noted between the amounts of radioactivity recovered in volatile-organic traps. However, as the grasses developed, the amount of radioactivity recovered was lower in vegetated chambers, resulting in significant differences by the end of the study (T=4.81, p=0.017; Table 1). Greater than 95% of the radioactivity

Table1. The distribution of radioactivity prior to starting the study, and following the study for unvegetated and vegetated chambers. Each value represents the percentage of the total recovered radioactivity for each measurement. Standard errors are shown in parenthesis (n=4). Asterisks indicate that vegetated chambers were significant different from unvegetated chambers at * $p<0.05$, and ** $p<0.01$.

| | Soil, Radioactivity Extracted with Ethyl Acetate | | | | Soil, Radioactivity Extracted With MeOH/Water | Soil, Residual Radioactivity After Extractions | Mineralized | Volatile Organic | Plant Tissue |
|---|---|---|---|---|---|---|---|---|---|
| | Total | Parent | Metabolites - Moderate Mobility | Metabolites- Low Mobility | | | | | |
| Pretest | 54.6 (1.1) | 40.7 (0.9) | 3.2 (0.2) | 11.5 (0.1) | 6.7 (0.3) | 38.7 (0.8) | -- | -- | -- |
| Unvegetated | 29.5 (0.5) | 17.6 (0.5) | 1.6 (0.1) | 10.2 (0.1) | 6.7 (0.3) | 54.9 (0.9) | 9.0 (0.5) | 0.48 (0.06) | -- |
| Vegetated | 26.2 (1.1) * | 14.7 (0.6) * | 1.8 (0.1) | 9.7 (0.5) | 7.8 (0.2)* | 55.3 (1.1) | 7.2 (0.3) ** | 0.29 (0.04) * | 3.1 (0.1) |

Table 2. Percentage change of pendimethalin residue in soil from pre-test values. Standard errors are shown in parenthesis (n=4). Asterisks indicate that vegetated chambers were significant different from unvegetated chambers at * $p<0.05$, and ** $p<0.01$.

| | Soil, Radioactivity Extracted with Ethyl Acetate | | | | Soil, Radioactivity Extracted With MeOH/Water | Soil, Residual Radioactivity After Extractions |
|---|---|---|---|---|---|---|
| | Total | Parent - Pendimethalin | Metabolites - Moderate Mobility | Metabolites - Low Mobility | | |
| Unvegetated, % Change from Pretest | 54.0 (1.4) | 44.2 (1.5) | 52.6 (7.7) | 89.0 (0.8) | 101 (8) | 142 (3) |
| Vegetated, % Change from Pretest | 48.2 (2.8)* | 37.0 (2.5)* | 61.2 (8.2) | 84.0 (4.5) | 118 (6)* | 143 (5) |

reported as volatile organic compounds was recovered from internal and external foam traps. Generally, ethylene glycol traps did not have measurable activity. Only a slight amount of radioactivity was recovered from the extraction of the polyethylene drying tubes that served as a holder for the foam traps (less than 500 dpm), indicating that they were not a significant sink for radioactivity. The internal traps collected 35% (SE 7) of the volatile organic compounds with no statistical difference between vegetated and unvegetated chambers. TLC evaluation of the combined volatile trap extracts for the final 36 days indicated that 91% of radioactivity in vegetated volatile traps and 84% of the radioactivity in unvegetated traps corresponded to pendimethalin.

Radioactivity was found throughout the plant. Overall, 3.1% (Table 3) of the total radioactivity in vegetated chambers was found in the plant tissue. Sixty percent of the radioactivity in the plant was recovered from the roots (Table 3). However, due to the larger mass of root, radioactivity equivalent to a pendimethalin concentration of 51.8 mg/kg (SE 4.6) was found in the root, while 89.6 mg/kg (SE 8.96) was found in the stem and leaf portion of the plant. Most of the radioactivity in the plant was not extractable by ethyl acetate. Furthermore, TLC fractionation indicated that only a small portion of the extractable material corresponded to pendimethalin (Table 3). Correspondingly, the pendimethalin concentration in stem and leaves was 1.03 mg/kg (SE 0.15), while the pendimethalin concentration in roots was 4.89 mg/kg (SE 1.05). Extractable radioactivity from stem and leaves distributed evenly up the TLC plates with the highest levels at the origin (metabolites with low mobility; Table 3). Extractable radioactivity from root was attributable primarily to pendimethalin and metabolites with low mobility.

**Discussion**

The primary goal of phytoremediation is to reduce the level of contamination in the matrix without causing other uncontained environmental contamination. In this study, vegetation significantly reduced the amount of pendimethalin in soil as compared to unvegetated controls (Tables 1 and 2). These findings are consistent with previously reported findings [13]. Although the differences due to vegetation are subtle when compared to the amount of pendimethalin loss that occurred over time, the presence of significant

Table 2. Percentage change of pendimethalin residue in soil from pre-test values. Standard errors are shown in parenthesis (n=4). Asterisks indicate that vegetated chambers were significant different from unvegetated chambers at * $p<0.05$, and ** $p<0.01$.

| | Soil, Radioactivity Extracted with Ethyl Acetate | | | | Soil, Radioactivity Extracted With MeOH/Water | Soil, Residual Radioactivity After Extractions |
|---|---|---|---|---|---|---|
| | Total | Parent - Pendimethalin | Metabolites - Moderate Mobility | Metabolites - Low Mobility | | |
| Unvegetated, % Change from Pretest | 54.0 (1.4) | 44.2 (1.5) | 52.6 (7.7) | 89.0 (0.8) | 101 (8) | 142 (3) |
| Vegetated, % Change from Pretest | 48.2 (2.8)* | 37.0 (2.5)* | 61.2 (8.2) | 84.0 (4.5) | 118 (6)* | 143 (5) |

differences indicates that if the process is better understood, improvement in technology may lead to a valuable remediation strategy.

Greater differences between vegetated and unvegetated treatments may have been present if the study had been continued for a longer period and a greater biomass to soil ratio was obtained. The biomass obtained was equivalent to 0.29 Mg/ha. For comparison, switchgrass grown for biomass obtains harvest levels of 6 to 19 Mg/ha [16]. The perennial grasses used in this study take more than one growing season to mature. Other than the length of study, low biomass obtained may have been due to slight herbicide injury due to the presence of pendimethalin in the soil.

Based on the extraction and separation techniques used in this study, only slight changes were noted regarding degradation of pendimethalin in soil due to the presence of prairie grasses. The amount of ethyl acetate-extractable metabolites and bound residues were not significantly different. However, the amount of radioactivity not extractable by ethyl acetate, but extractable by methanol/water, was significantly different between vegetated and unvegetated soils. Since the primary change in the soil distribution of radioactivity involved only two of the measured groups (a drop in pendimethalin and an increase in methanol/water-extractable products) vegetation may change specific degradation pathways instead of causing a more general increase in all degradation pathways. The difference between vegetated and unvegetated chambers in methanol/water extraction was an increase of 1.1% of the total recovered radioactivity in vegetated columns. The magnitude of the difference in pendimethalin recovered from the soil was a decrease of 3.3% of total recovered radioactivity, indicating that this degradation pathway is only part of the difference in the fate of pendimethalin residues.

Pendimethalin can be transformed into numerous products. Photodegradation alone can result in nine major and three minor products. Both photodegradation and microbial degradation result in partial and full dealkylation of the substituted amine, reduction of nitro groups, cyclization to benzimidazole products, and ring hydroxylation [17, 18, 19, 20]. Little work has been conducted investigating the extractability from soil, environmental fate, or toxicology of the metabolites. Since it was not feasible for determination of metabolites in this study, further work needs to be conducted to identify the metabolites and the environmental consequences of a greater concentration of metabolites.

In both vegetated and unvegetated chambers, approximately 55% of the radioactivity recovery was from bound residues. Bound residue in this study was defined as unextractable by ethyl acetate and by a methanol/water solution. Pendimethalin has been shown to form bound residues in soil in other studies. For example, Barriuso et al. [21] reported 45% of applied radioactivity as bound residues in soil after 240 days and 53-55% as bound residues when compost was added to the soil. In another study, Nelson et al. used an acetone:$H_2O$:HCl (95:4:1) extraction and reported 15% of applied radioactivity bound after 180 days [22]. In our study, higher recovery may have been possible with a strongly acidic solution such as used by Nelson et al. [22]; however, the hydrolysis potential of this extraction technique would result in a different working definition of a bound residue.

Mineralization of pendimethalin and pendimethalin-derived residues was lower in vegetated chambers (Table 1). The decrease could be the result of two processes; either the microbial population shifted in vegetated columns so that full mineralization occurred at a lower rate, or some of the $^{14}C$-$CO_2$ was incorporated into the plant. The high flow rate in the chambers (air turnover every 13 minutes) likely prevented substantial incorporation of $^{14}C$-$CO_2$. The degree of mineralization found in this study (7-9% of applied radioactivity after 108 days) is similar to the reported values for lysimeter surfaces (3-5 % of applied in 36 days)[23]. Another study reported that a higher degree of pendimethalin mineralization in soil (42%, 240-day test), and a degree of mineralization similar to what found in this study, when compost was added to the soil (16-24%) [21].

The amount of radioactivity recovered in volatile traps also decreased in vegetated chambers. This decrease could also be the result of more than one process, including adsorption of volatile compounds (including pendimethalin) by the plant or changes in soil structure reducing volatilization. The lack of radioactivity recovered by swiping the grass at the end of the study indicates that any adsorbed pendimethalin was able to enter the plant. The degree of pendimethalin volatilized (0.3-0.5% of applied in 108 days) was lower than previously reported in studies for lysimeter surfaces (1.2 to 2.8 % in 28 days)[23]. This difference is likely due to the high level of incorporation of pendimethalin into the soil and the aging process prior to the start of the study. Although plastic materials were used to build the chambers, traps, and tubing, efforts to extract radioactivity out of the trap casing and swipes of the chamber itself yielded negligible radioactivity. The radioactivity recovered

from volatile traps was primarily attributable to parent pendimethalin indicating that dissipation of radioactivity from the soil and into air is mostly due to direct pendimethalin loss rather than production of volatile metabolic products.

Despite the low water solubility (0.3 mg/L) and high soil adsorption coefficient ($K_{oc}$=7011 ml/g in loamy sand, 0.87% OC) of pendimethalin [24], over 3% of the recovered radioactivity was found in the vegetation (Table 1). This level in vegetation may indicate that direct uptake is an important factor in increasing soil dissipation. Recent phytoremediation studies involving DDE resulted in contaminant extraction rates of 0.40 to 2.4% (percentage of soil contaminant found in the plant) [25]. In a study by Brown et al. [26] a heavy metal accumulating species, *Thlaspi caerulescens*, removed 0.6 to 1.3% of of Zn and Cd from soil. Although pendimethalin is not as recalcitrant as heavy metals or DDE, and thus the studied system was not at steady-state concentrations, this level of uptake in the plant could be useful in increasing pendimethalin degradation rates, especially if the rate remains consistent (or increases) as biomass increases. Other organic compounds with higher soil mobility have higher uptake into the plants. Poplar trees have been shown to take up 20-30% of radiolabelled atrazine applied to soil [27].

Only a small percentage of the radioactivity found in the plant was attributable to pendimethalin (Table 3). Instead primarily unextractable residues were present, indicating that either pendimethalin is highly metabolized in the plant or that radiation in plant material was primarily due to uptake of polar pendimethalin metabolites. If much of the radioactivity in the plant was from metabolite uptake, then the original dissipation route of the pendimethalin would be through degradation in the root zone (rhizodegradation). Uptake of degradation products is also an important mechanism decreasing the potential for environmental damage due to metabolites.

Other paths of uptake of radioactivity into the plant include uptake of $^{14}C$-$CO_2$ and incorporation into biomass, and adsorption and uptake of volatile organics through the leaf and stem. The amount of volatile compounds within the system was very low in both systems (Table 1). Although the amount was lower in vegetated systems, the difference was only 0.19% of recovered – not enough to greatly influence the magnitude of plant uptake. The amount of $^{14}C$-$CO_2$ collected was also lower in vegetated chambers. However, the high

turnover rate of the air in the chamber reduces the chance of uptake into stem and leaf material.

Much higher concentrations of pendimethalin were found in the roots as compared to the stem and leaves; however, higher concentrations of radioactivity per mass of plant were found in the stem and leaves (Table 3). Pendimethalin and other dinitroanaline herbicides are generally not considered as systemic herbicides and are not usually reported to translocate in plants [28]. However, metabolites of $^{14}$C-trifluralin, a related dinitroanaline herbicide, were shown to accumulate in carrot tops (0.283 mg/kg based on total radioactivity) [29]. In this study, the concentration of pendimethalin found in the stem and leaf material was 1.03 mg/kg. This level is above the EPA maximum allowable level in most commodities of 0.1 mg/kg [24]. The high level found in the leaf material is due to the high soil concentrations used in the study. If residues of this type are to be phytoremediated, the resulting vegetation may not be suited for feeding livestock, and further evaluation is needed to determine if there would be a risk to wildlife. However, plant tissue may not need to be treated as hazardous waste. As plant material is returned to soil, pendimethalin and pendimethalin metabolites within the plant may further degrade as the plant material decays. A life cycle assessment of this residue would more fully account for the degradation and location of the pesticide as the phytoremediation system ages and plant material turns over.

The chamber design worked well for this mass-balance study. The mass balance averaged 83% for unvegetated and 87% for vegetated chambers. These values are similar to those found in other studies. For example, atrazine mass balance in enclosed systems ranged from 83-100% [27] and a study conducted on the explosive RDX reported 80% mass balance [30]. It is unclear as to why unvegetated chambers had a lower mass balance. However, the difference was not enough to affect the data when represented as the percentage of recovered radioactivity.

Aging of the pendimethalin residues in the soil, prior to starting the study, provided a more realistic evaluation of the remediation technique for point source contamination such as an aged-pesticide spill at agrochemical dealership. However, degradation products formed during the aging procedure reduce our understanding of pendimethalin uptake into prairie grasses. Although the manufacturer of pendimethalin has presented considerable information about plant uptake and transformation of pendimethalin to the U. S. Environmental

Protection Agency [24], very little information is available in peer-reviewed literature. Further data, using basic plant-uptake method designs, are needed to fully understand the role plant uptake may have on phytoremediation of pendimethalin.

Vegetation, and specifically prairie grasses, may be valuable in management practices for reduction of herbicide contamination. In this study, pendimethalin dissipation was increased in vegetated soil through uptake into the plant and enhanced degradation in the rhizosphere. Further understanding of these processes may improve phytoremediation technology.

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# CHAPTER 5 . ENVIRONMENTAL HAZARD EVALUATION OF PENDIMETHALIN CONTAMINATED SOIL

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**A paper to be submitted to *Archives of Environmental Contamination and Toxicology***

## Abstract

The effects of pendimethalin on seedling growth, earthworm growth, springtail reproduction, and pill bug survival were determined. Additionally earthworm accumulation factors were determined and used with plant accumulation factors available from the literature, to calculate allowable soil concentrations that would not cause toxicity to rodents and birds following trophic transfer from contaminated soil. Pendimethalin significantly impacted seedling growth of four plant species at levels below 10 mg/kg, reduced earthworm growth at 10 mg/kg, and decreased springtail reproduction at 90 mg/kg. No lethal effect was found on pill bugs. Soil concentrations as low as 30 mg/kg may have the potential to cause bird toxicity as a result of trophic transfer through earthworms. Rodents would probably be less vulnerable. Overall, even low soil concentrations (less than 10 mg/kg) could result in reduced productivity and diversity in the contaminated soil, higher concentrations (30 mg/kg and above) could impact the surrounding environment due to trophic transfer through earthworms.

## Introduction

Pendimethalin, N-(1-ethylpropyl)-3,4-dimethyl-2,6-dimethyl-dinitrobenzeneamine, is an extensively used dinitroanaline herbicide. It is used on a large number of agricultural and nonagricultural sites worldwide for control of broadleaf weeds and annual grasses. In 1997, 23-27 million pounds of pendimethalin were applied to agricultural sites in the United States with the majority used on soybean, cotton, and corn. Another 2-3 million pounds were applied to nonagricultural sites within the United States primarily on lawns (EPA 1997). Dinitroanaline herbicides act as microtubule disruptors in plants (Rao 2000) and protozoa

(Arrowood et al. 1996) by inhibiting polymerization of tubulin. In plants, inhibition of growth occurs because mitosis is disrupted. However, due to limited uptake and distribution by plants, inadequate root growth is the primary toxic effect (Rao 2000). Dinitroanalines are not reported to disrupt mammalian microtubules (EPA 1997); however, research regarding the effects of pendimethalin on invertebrates is very limited.

Pendimethalin and other pesticides can become environmental contaminants in two ways, they can be applied to fields, followed by movement into physical or biological compartments other than those intended for pest control, or they can be accidentally spilled in areas not intended for treatment. Although most research and risk assessment efforts are focused on potential environmental effects following field applications, incidental and accidental spillage has become a growing concern. For example, studies have estimated that 90% of agrochemical dealerships in Iowa (USA) contain some level of pesticide-contaminated soil, and 50% of those will require some degree of remediation (Gannon 1992). Contamination due to spillage at agrochemical dealerships often occurs at concentrations well above intended field applications.

The half-life of pendimethalin in soil can be very long; at 10° C the half-life for pendimethalin was reported to be over 400 days (Walker and Bond 1977). In an experiment conducted in our laboratory, soil contaminated with over 100 mg/kg pendimethalin through incidental spillage at an agrochemical dealership, was incubated at greenhouse conditions. After 1,026 days, greater than 40-60% of the pendimethalin remained in the soil (Belden et al. 2003a). Pendimethalin is one of the only current use pesticides to be placed on the United States Environmental Protection Agency's Persistent, Bioaccumulatory, and Toxic list as part of the Toxic Release Inventory (EPA 1999).

The extensive use of pendimethalin, coupled with high persistence, make it a likely soil contaminant. Soil contaminants can harm the environment in three primary ways. 1] The contaminant may adversely affect organisms that live within the contaminated soil. 2] The contaminant may move from the contaminated soil into biota at concentrations that could adversely affect the surrounding environment. 3] The contaminant may move from the contaminated soil into surface and groundwater. Since, pendimethalin has little mobility after incorporation into soil (Zheng et al. 1993; Belden et al. 2003b) it is of limited concern in regards to surface and groundwater (EPA 1997). However, limited research has been

published in regards to pendimethalin toxicity to soil organisms or uptake into the terrestrial food chain through soil exposure, and even less research has been conducted on pendimethalin concentrations above those expected after field application.

In this study, we selected three prairie grass species that are potentially useful in remediation and restoration of pesticide-contaminated sites (big bluestem, indian yellowgrass, and switchgrass; Belden et al. 2003B) and lettuce, a common test species, and conducted seedling growth assays. Seedling growth was chosen as a sensitive indicator of toxicity due to the mode of action of pendimethalin. Three common soil invertebrates (springtail, earthworm, and pill bug) were also selected based on their importance in soil for turnover of decaying plant material and other soil processes. Standard reproduction endpoints were used to evaluate toxicity to springtails, while early-life stage growth was chosen for the primary endpoint in earthworm assays. Both are likely to be sensitive indicators of pendimethalin toxicity. Lethality was used as the primary endpoint during pill bug tests, since less is known about their biology and susceptibility to pesticides. Toxicity tests were conducted with each organism using pendimethalin concentrations at and above field application levels in order to estimate the soil concentration of pendimethalin that would impact soil organisms adversely. In addition, bioaccumulation factors for earthworms were determined based on body burdens obtained during the 21-day growth study. These factors, along with prairie grass bioaccumulation factors determined in a different study, were used to estimate the toxicity risk of pendimethalin to birds and rodents through trophic transfer.

## Methods

### *Chemical and Reagents*

Technical grade pendimethalin was obtained in kind from American Cyanamid (currently BASF, Research Triangle Park, NC, USA). It was purified to greater than 98% purity by column chromatography using silica gel as a stationary phase and hexane:acetone 95:5 as a mobile phase. Purity was checked based on gas chromatography coupled with flame ionization detection and by high-pressure liquid chromatography coupled with ultraviolet absorption detection. All solvents used were of HPLC or pesticide grade, and all other reagents were similarly of high purity.

*Organisms*

Big bluestem (*Andropogon gerardii*; Pawnee variety), yellow indiangrass (*Sorghastrum nutans*; Holt variety), and switchgrass (*Panicum vergatum*; Pathfinder variety) seed was purchased from United Seed Company (Omaha, NE, USA). Lettuce seeds (*Lactuca sativa*) were obtained from Carolina Biological (Burlington, NC, USA). Purity for all seeds was greater than 97%. Seeds were stored at room temperature for less than three months prior to use.

Springtails (*Folsomia candida* Willem) cultures were obtained from Oklahoma State University, Ecotoxicology and Water Quality Laboratory (Stillwater, OK, USA). The organisms originated from Dr. Renata Snyder, Michigan State University (Lansing, MI, USA), who collected the original stock of 30 organisms in a Michigan cornfield (personal communication). They were cultured in our laboratory for two years prior to toxicity testing. Culturing was performed, as previously described (Wiles and Krogh 1998), on a hydrated $CaSO_4$, activated-charcoal medium using baker's yeast as a food source. Five weeks prior to the start of the study, 50 adults were placed in a container and allowed to lay eggs. After four days, the adults were removed. The resulting offspring developed to a reproductive state prior to the start of the toxicity test. Since egg hatch completion is 14 days in this species, the test organisms were approximately 20 days old, the approximate age of reproductive maturity. This parthenogenic organism can live up to 290 days, continuing to reproduce their entire adult life (Wiles and Krogh 1998).

Earthworms (*Eisenia fetida*) were acquired from a local vender and cultured for over three years prior to these bioassays. Culturing was conducted by adding 10 adults each to 3-L square containers filled with a mixture of potting soil and horse manure (collected from a horse known to not be medicated). As containers became populated with earthworms, new containers were started. Five weeks prior to starting the earthworm bioassays, approximately 50 cocoons (egg cases) were removed from three different culturing containers and placed in potting soil in a fresh container. Cocoons produce an average of 3.4 juveniles after three weeks of development (Tomlin and Miller 1980). At test time, young worms, 7-20 mg in size were collected from this container. Due to the long hatch time, three weeks difference in hatch time may be possible; however, since neither the largest or smallest worms were collected, this difference was minimized.

Pill bugs were wild–caught in a greenhouse bay at Iowa State University that had not received pesticide treatment for longer than four years. Captured organisms were divided based on gross morphological characteristics. A large number of organisms with similar morphology were identified to be in the genus *Armadillidium*. Pill bugs were held in captivity greater than 45 days prior to use in bioassays. During this time they were kept in a glass aquarium with potting soil as a substrate and raw potato slices for a food source.

*Toxicity Test Methods*

Each bioassay was conducted using agronomic soil from a reference site that has not received pesticide usage in over 10 years (Field 55, Iowa State University Agronomy-Ag Engineering Research Farm, Ames, IA, USA). The soil was sieved (2.8 mm) to remove plant material and rocks. Analysis of the soil indicated a sandy loam texture (60% sand, 22% silt, and 18% clay), 2.7% organic matter, and a pH of 7.0 (Midwest Laboratories, Omaha, Nebraska, USA). Field capacity was determined to be 17% (water/dry soil). Soil was allowed to air-dry to greater than 97% solid, followed by moistening to near field capacity, and then allowed to air-dry again. This procedure was intended to reduce soil fauna to prevent competition or predation of the test organisms.

In order to determine toxicity to plants, 14-day seedling growth assays (prairie grasses) and 7-day growth assays (lettuce) were performed following general standardized guidelines for plant toxicity tests (ASTM 1994). Twenty grams of soil was placed into 7.0 x 4.0-cm glass jars (approximately 3-cm soil depth). Soil was treated within each jar with 100µl of spiking solution (acetone as a carrier) to obtain pendimethalin concentrations of 0, 1, 4, 64, 256 mg/kg. Moisture was adjusted to field holding capacity, and 10 seeds were planted in each jar. Forceps were used to place the prairie grass seeds 0.5 cm deep. Lettuce seeds were left on top of the soil, exposed to light. The top was covered with plastic wrap and four small holes were inserted using a pin. Throughout the study moisture was added as necessary based on a gravimetric basis. At the end of the study, seedlings were carefully removed from the soil and length of the entire seedling was measured, and germination rates were noted. Three replicates were performed per treatment.

In order to determine toxicity to soil invertebrates, soil, 8 g for springtails, 12 g for pill bugs, and 25 g for earthworms, was added to 3.5 x 4.0-cm glass jars equipped with plastic lids containing two 1.6-mm holes to allow air exchange. The soil was treated with 100µl of a

pendimethalin solution in acetone to achieve 0, 10, 30, and 90 mg/kg for springtails, 0, 10, 40, 160 mg/kg for earthworms, and 0, 50, 100, 200 mg/kg for pill bugs. The soil was mixed inside the jar and the jars were left open overnight to allow volatilization of acetone. Twenty replicates were conducted for each treatment level.

Springtail tests were conducted similarly to standardized tests (ISO 1994), moisture in test chambers was adjusted to field capacity, and three granules of baker's yeast were added. Since this species is parthenogenic, a single springtail was added to each jar. The test lasted for 28 days at 20° C and a 16:8 light:dark cycle. Brown paper separated the test chambers from the light source, thus reducing light exposure. During the test, moisture and food were checked every 72 hours. Moisture was maintained at initial levels based on mass of the container and yeast was added if needed and replaced if uneaten. Every 3-4 days, adult survival and presence of offspring was recorded. At the end of the study, soil was gently flooded with reagent water saturated with sucrose. The container was exposed to ethyl acetate fumes to anesthetize the springtails and then the springtail populations were counted under a magnifying glass.

Earthworm studies were conducted following the general guidelines recommended within standardized methods (ASTM, 1998). However, the primary endpoint was different from what is generally measured. Most studies either measure 14-day acute toxicity in adults, adult weight loss, or adult reproduction success (Kula and Larink 1998). In this study, juvenile growth was chosen since it is likely a sensitive endpoint for pendimethalin and it is less intensive to conduct as compared to adult reproductive assays. Soil moisture was adjusted to 130% of field capacity (22% water/dry soil; 75% of water holding capacity). A single worm (7-20 mg) was dipped in reagent water, blotted dry with a paper towel, weighed to the nearest 0.1 mg, and added to a jar. A fine layer (50 mg) of cerophyll (ground cereal leaf material) was applied to the top of the soil every seven days (in total 0.75% of the soil mass). Test chambers were kept at 23° C, in the dark. Although standard methods for earthworm testing suggest 24-hour light (ASTM 1998), a more natural dark environment was used in this study. During the test only two worms were noted out of the soil, both in the highest treatment level. After 21 days, the degree of soil disruption was noted as either total disruption of surface or not. The earthworms were dipped in reagent water, blotted dry with a paper towel, and weighed. In addition, an aliquot of soil from earthworm bioassays pre- and

post-test was mixed with an equal mass of deionized/reverse osmosis-treated water to determine soil pH.

For measurement of pill bug lethal effects, a single pill bug was added to each test container, and moisture was adjusted to field capacity. Each pill bug was fed a rectangular piece of fresh, green corn leaf (0.5 x 1.0 cm). At 3-4 day intervals, moisture in each container was adjusted to field capacity, and the food was replaced. Consumption of corn was noted based on disruption of the margins of the corn leaf – any disruption was considered a positive response. Survival and the position of the pill bug in the soil (under the soil surface versus on the soil surface) also were noted. Additional information, such as molting and reproduction, was noted. After 14 days, pill bugs were removed and observed for the ability to unroll from the ball position and move their legs; pill bugs unable to do this were recorded as dead.

*Measurement of soil concentrations*

One additional test chamber was constructed for each treatment in each test to provide soil for analysis and verification of pendimethalin concentrations. In addition, after each test, soil from several test containers was combined to form a single sample for each treatment in each test. Each soil sample was placed in 200-ml jars with 4:1 by volume of ethyl acetate: mass of soil, which was shaken vigorously for 20 minutes. The solvent was removed and filtered, and the process was repeated twice. The combined extracts were evaporated to a 5-ml final volume under a stream of nitrogen. The extract was analyzed by gas chromatography coupled with thermionic specific detection (GC-TSD, nitrogen and phosphorous specific; Varian 3400, Walnut Creek, CA). Extraction efficiency for this method was 95% (SD 11) for pendimethalin.

*Measurement of earthworm BAF*

Earthworms from the toxicity tests were stored at -4°C following the toxicity experiment. They were extracted with 10 ml of acetone in a sample homogenizer followed by two additional extractions with 10 ml ethyl acetate. The combined extract was filtered, dried with anhydrous sodium sulfate, concentrated to 5 ml, and analyzed using GC-TSD as previously described. Biological accumulation factors (BAFs) were calculated as concentration in the earthworm/concentration in soil.

*Hazard evaluation for birds and rodents due to trophic transfer of pendimethalin*

Earthworm bioaccumulation factors determined in this study, prairie grass bioaccumulation factors reported in a previous study (Belden, 2003), toxicity to model organisms (rat and quail; EPA, 1997), and food consumption rates of selected non-target birds and mammals (EPA, 1993) were used to estimate soil contamination limits. Risk quotients (RQ) were also included in the calculations to provide more conservative tolerable soil contamination limits that will reflect potential intra and interspecies variations. An acceptable RQ of 0.5 (effective concentration/ environmental concentration) was used for mammals and a higher acceptable RQ of 0.1 was used for birds. The bird RQ was necessarily higher due to the lack of acceptable chronic toxicity tests conducted for birds (EPA 1997). Soil contamination limits were calculated as follows (Stephenson et al. 1997):

*Daily dose = Concentration in Soil x BAF x Food Consumption Rate*

The effective concentration (NOEL for example, mg/kg/day) can be multiplied by the risk quotient to obtain a limit for the acceptable daily dose. If this is substituted for the *daily dose*, the correct *BAF* reflecting the uptake from soil into the food source is inserted, and the *food consumption rate* of the organism of interest is inserted. The *concentration in soil* that represents the tolerable soil concentration can be determined algebraically. To provide conservative tolerable soil concentrations, the most conservative values available were inserted into the formula. Table 2 provides the input information.

*Statistical design and analysis*

For each toxicity test, experimental units were randomly assigned numbers 1-80. Each number corresponded to the order in which organisms were added, position in the environmental chamber, and in which order measurements were made. Analysis of variance (ANOVA) was used in combination with Fisher's PLSD ($p<0.05$) to determine if pendimethalin had a significant effect and which concentrations differed from controls for continuous data. Categorical data were analyzed using Chi Square analyses using the control as the expected outcome. Computations were made using Statview for Windows (1998). Inhibition concentrations and effective concentrations were determined using the Trimmed Spearman-Karber method (Hamilton et al. 1977). Standard errors (SE), or standard deviation (SD) where appropriate, are reported in parentheses following all mean values.

**Results and Discussion**

Pendimethalin significantly inhibited seedling growth for all species tested ($F=17$ to 112, $p<0.001$). Figure 1 shows the dose-response curves for each species. The highest concentration not significantly different from the control is reported in Table 1 as the NOEC and the lowest concentration that was significant is reported as the LOEC ($p<0.05$). For both big bluestem and yellow indiangrass, the lowest concentration tested elicited a significant effect ($p<0.05$) preventing determination of a NOEC. Switchgrass and lettuce were less sensitive than yellow indiangrass and big bluestem; however growth in all species was inhibited at concentrations less than 10 mg/kg. This concentration represents the highest application rate of pendimethalin (4 kg/ha) distributed through the top 4 cm of the soil column. Due to the low mobility of pendimethalin, this is a worst-case, although obtainable, concentration due to normal application.

Germination rates were greater than 90% for all lettuce treatments up to concentrations of 64 mg/kg. At 256 mg/kg germination did not occur. Germination rates in the low level prairie grass treatments (1 and 4 mg/kg) were variable ranging from 60-90% and were not significant different from controls. Due to the low germination rate in prairie grasses, LOEC and NOEC values were not considered valid for this endpoint. Complete inhibition of germination occurred for yellow indiangrass and big bluestem at 16 mg/kg and at 64 mg/kg for switchgrass.

Other researchers have found similarly high toxicity for other plant species. Plant height of ryegrass was inhibited by 25% at 0.10 kg/ha, and onion survival was reduced 25% by 1.4 kg/ha (EPA 1997). However, researchers investigating potential use of the prairie grass species for phytoremediation have successfully transplanted plugs of grass into soil containing over 100 mg/kg of aged pendimethalin residues (Belden 2003d). With effort, tolerant species may be transplanted into soil moderately contaminated with pendimethalin.

Pendimethalin significantly reduced the percentage of springtails capable of

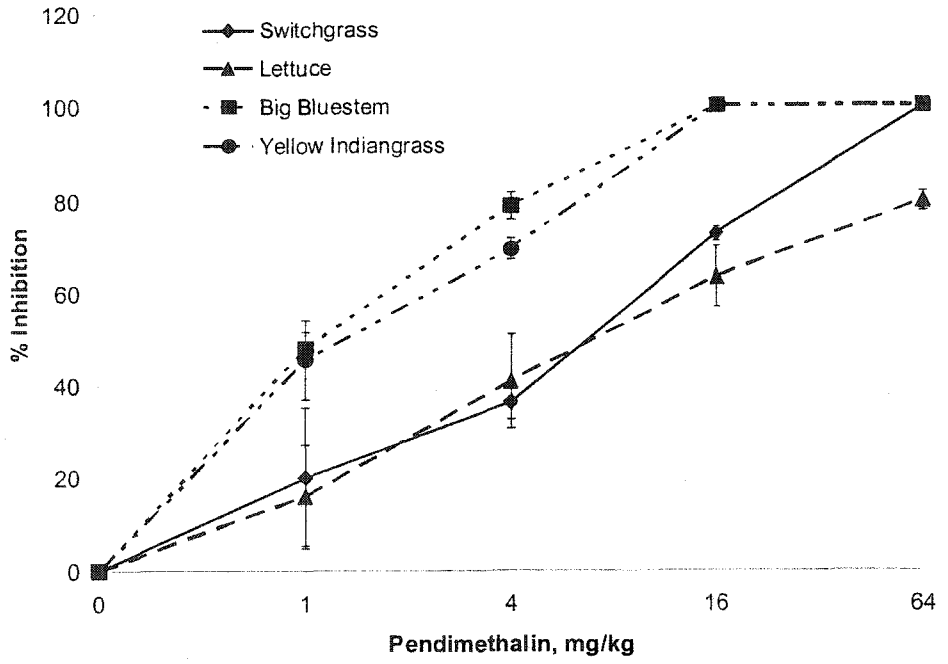


Figure 1. Effect of pendimethalin on seedling growth. Error bars represent one standard error.

reproducing (for the highest treatment compared to control, $X^2$ =33, $p<0.001$; no other treatments were significant) and the number of offspring produced per springtail (F=25, $p<0.001$). Both the LOEC and the NOEC are shown in Table 1. The $IC_{50}$ for both endpoints was also nearly identical, measuring at 54 mg/kg for percentage reproducing and 47 mg/kg for number of offspring produced. Figure 2 provides the percentage of springtails that had reached reproductive status at time points throughout the experiment, and Figure 3 provides the mean number of offspring recovered at the end of the experiment for each treatment. For both endpoints, little to no effect occurs at concentrations up to 30 mg/kg followed by a dramatic decrease in reproductive success at 90 mg/kg. The time necessary for reproduction to occur may have been delayed in the 30 mg/kg, treatment as indicated by the low reproduction rate at 14 days. However, by 17 days this treatment was equal to the control group. No mortality was observed in any springtail treatments despite the fact that springtails in the highest concentration became yellow, presumably due to uptake of pendimethalin which is highly yellow/orange colored. Little research has been published

Table 1. Toxicity of pendimethalin to selected soil organisms.

| *Species* | *Length of Test* | *Endpoint* | *NOEC*[a], *mg/kg* | *LOEC*[b], *mg/kg* | *$IC_{50}$ or $LC_{50}$*[c], *mg/kg (95% C.I.)* |
|---|---|---|---|---|---|
| Lettuce *Latuca sativa* | 7 day | Seedling growth | 1.0 | 4.0 | 7.74 (5.67-10.57) |
| Switchgrass *Panicum vergatum* | 14 day | Seedling growth | 1.0 | 4.0 | 6.23 (4.68-8.28) |
| Big Bluestem *Andropogon gerardii* | 14 day | Seedling growth | NC[d] | 1.0 | 1.09 (0.72-1.66) |
| Yellow Indiangrass *Sorghastrum nutans* | 14 day | Seedling growth | NC | 1.0 | 1.33 (0.84-2.13) |
| Springtail *Folsomia candida* | 28 day | Reproduction-Number offspring produced | 30 | 90 | 47 (44-51) |
| Earthworm *Eisenia fetida* | 21 day | Growth-Early Life Stage | NC | 10 | 113 (84-115) |
| Pillbug *Armadillidium* | 14 day | Survival | 200 | NC | >200 |

[a]NOEL- no observable effects concentration. [b]LOEL- lowest observable effects concentration. [c]$IC_{50}$- concentration necessary to cause a 50% inhibition of growth or reproduction or $LC_{50}$- concentration necessary to cause a 50% reduction in survival. C.I.-confidence interval. [d]NC- not calculated due to inadequate data. [e]ELS- early life stage test.

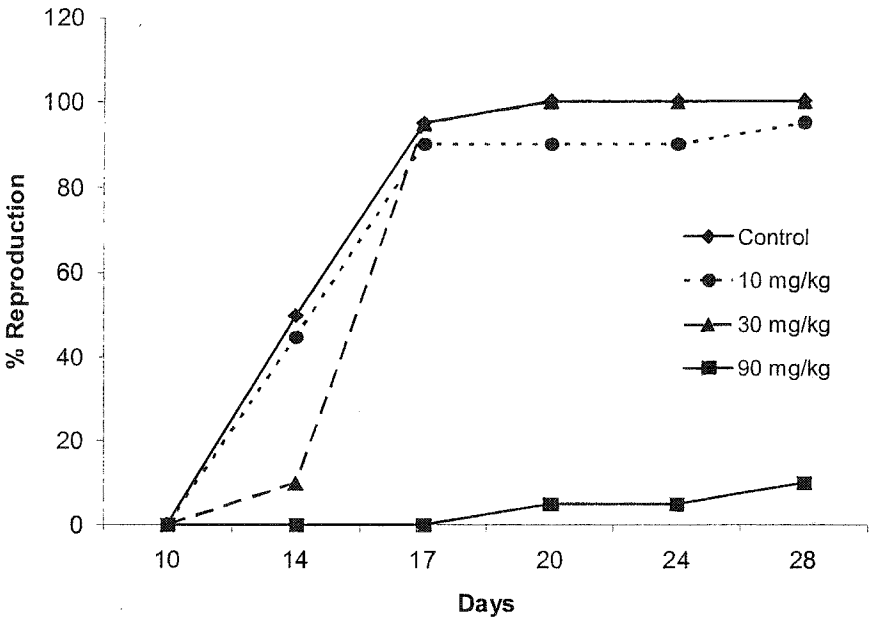


Figure 2. Percentage of springtails to exhibit reproduction during the study.

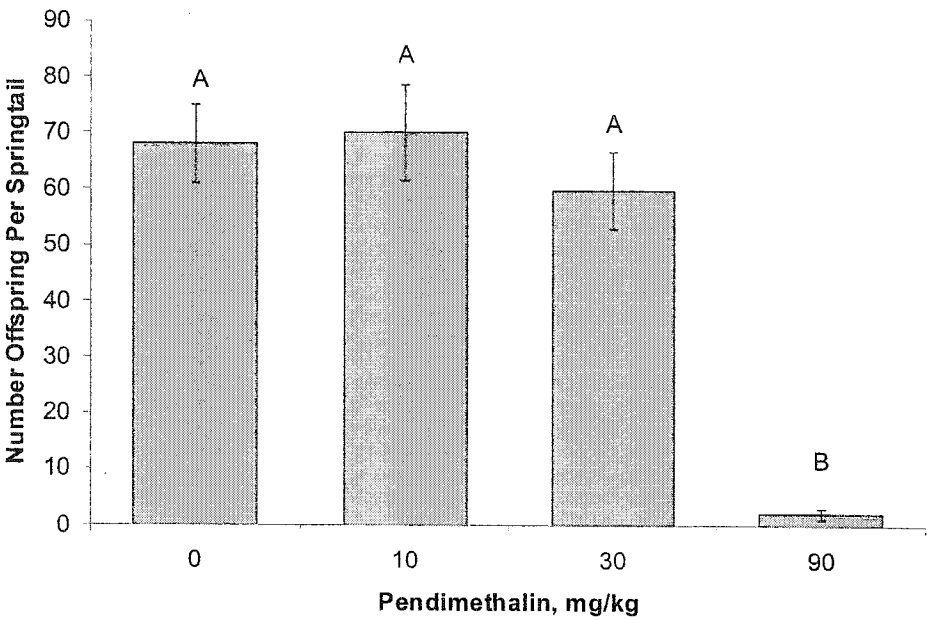


Figure 3. Effect of pendimethalin on the number of offspring produced per springtail. Treatments not marked with the same letter are significantly different ($p<0.05$). Error bars represent one standard error.

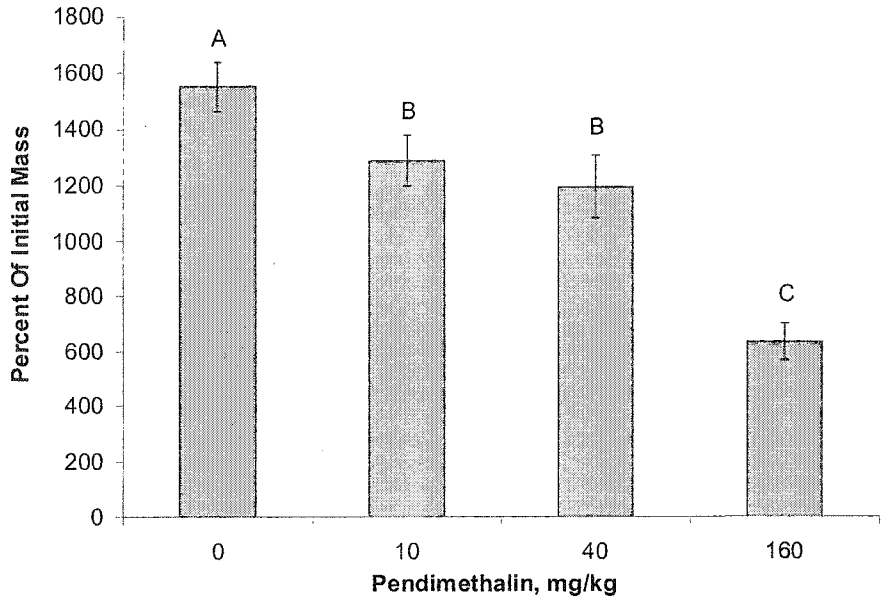


Figure 4. Pendimethalin significantly reduced weight gain of early life stage earthworms (*Eisenia fetida*). Treatments that are not marked with the same letter are significantly different ($p<0.05$). Error bars represent one standard error.

regarding the chronic toxicity of herbicides to springtails. However, in a study measuring the acute toxicity of insecticides to *Folsomia candida*, $LC_{50}$ values were widely spread, ranging from greater than 50 mg/kg (DDT) to less than 0.05 mg/kg (phorate; Thompson and Gore 1972).

Earthworms obtained substantial growth during the 3-week study with an average increase in controls of over 1500%. Pendimethalin significantly decreased weight gain ($F=17$, $p<0.001$); even the lowest treatment, 10 mg/kg, it was significantly different from the control ($p<0.05$; Figure 4). The NOEC, LOEC, and $IC_{50}$ are shown in Table 1. No mortality was found in the control or the 10 mg/kg treatment. Five and ten percent mortality was recorded in the 40 and 160 mg/kg treatments, respectively. Since 10 mg/kg could be obtained during normal application, some concern may be warranted in regards to the impact of pendimethalin on earthworm populations after application using the highest labeled rates. However, it is unclear whether any population decreases would occur due to a 17% decrease

in weight gain. In addition, the degree of soil processing was also measured as a subjective evaluation of earthworm feeding and activity. This endpoint was less sensitive than growth. The 10 mg/kg treatment was not significantly different from controls (80-85% disturbed). At 40 mg/kg, significantly fewer experimental units had completely disturbed soil (58%, $X=5.9$, $p=0.02$), and at 160 mg/kg only 22% of the soil had been disturbed ($X=13$, $p<0.001$).

Pill bugs were not significantly affected by pendimethalin up to concentrations of 200 mg/kg. Survival rate in the controls and lower concentrations was 100%. Survival in the 200 mg/kg treatment was 85%. No differences were noted in the percentage of pill bugs that were actively eating (78% of measurements) or burrowing (63% of measurements). Out of the 80 organisms in this study, eight molted in the two-week period and three reproduced successfully. One of the successful reproduction events occurred in a 200 mg/kg treatment, the young appeared to be healthy.

Although these data show that some soil invertebrates (earthworms and springtails) may be adversely affected by pendimethalin, the high toxicity of pendimethalin to plants may cause greater population effects due to changes in the amount of vegetative debris in the soil. Other studies have shown that although herbicides may impact earthworm populations, the type and amount of plant residues may have a greater effect (Farenhorst 2003).

Accumulation of pendimethalin in earthworms resulted in BAF values ranging from 1.1 at 10 mg/kg, 1.4 (SE 0.2) at 40 mg/kg, and 0.75 (SE 0.05) at 160 mg/kg. An earlier study, using the same soil, reported an 8-day BAF for pendimethalin of nearly 2 (Belden et al. 2003b). This same trend of decreasing BAF values over time was found between 7 and 21-day treatments when *Lumbricus terrestris* was exposed to diazinon (Stephenson 1997). This trend may be due to decreasing bioavailability during the study. The highest 21-day value was used for trophic transfer investigations. Accumulation studies were conducted without allowing for gut clearance. Although this may make the results more variable, it is more applicable for trophic transfer investigations.

Table 2 shows the calculated soil tolerances based on potential toxicity to birds and rodents. The avian species feeding on earthworms were the most vulnerable with tolerable soil limits around 30 mg/kg, just three times above the highest application levels. The low levels are partially due to the 10x safety factor (0.1 RQ) used for calculations. However, conservative estimates are needed due to the lack of chronic toxicity data available for birds.

Table 2. Tolerable soil concentrations based on rodent and avian exposure due to trophic transfer of pendimethalin from contaminated soil.

| Organism | Dietary Exposure Parameters | | | Toxicological Endpoint Parameters[A] | | | | Tolerable Soil Concentration, mg/kg |
|---|---|---|---|---|---|---|---|---|
| | Food | BAF (Food/Soil) | Food Consumption Rate, g/g/day[B] | Reference Test Species | Endpoint | Acceptable RQ[C] | NOEL, mg/kg/ day | |
| Prairie Vole (*Microtus ochrogaster)* | Grass stem and leaf | 0.15[D] | 0.14 | Rat | Reproduction | 0.5 | 172 | 4100 |
| Prairie Vole (*Microtus ochrogaster)* | Grass root | 0.75[D] | 0.14 | Rat | Reproduction | 0.5 | 172 | 820 |
| Northern Short-Tailed Shrew (*Blarina brevicauda*) | Earthworm | 1.4 | 0.62 | Rat | Reproduction | 0.5 | 172 | 99 |
| American Robin (*Turdus migratorius*) | Earthworm | 1.4 | 0.89 | Northern Bobwhite Quail | Subacute Lethality | 0.1 | 326 | 26 |
| American Woodcock (*Scolopax minor*) | Earthworm | 1.4 | 0.77 | Northern Bobwhite Quail | Subacute Lethality | 0.1 | 326 | 30 |

[A]Parameters obtained from reference EPA 1997.
[B]Highest values listed in reference EPA 1993.
[C]Acceptable risk quotient is more conservative for avian species since chronic endpoints are not available.
[D]Plant BAF based on a study using a mixture of prairie grass and lasting 108 days. Soil concentrations decreased throughout the study. The final soil concentration was used to calculated BAF values as a conservative estimate (Belden 2003c).

Rodents are less vulnerable with tolerable soil levels 99 mg/kg and above. Herbivores were the least sensitive due to the low uptake into plants (especially leaf and stem, and likely, seeds). However, some herbivores may have a higher food consumption rate, which would decrease the tolerable soil concentrations. The toxicological endpoint used for rodents was based on reproduction studies following work by the EPA for ecological risk assessment of pendimethalin (EPA 1997); however, lower NOEC and LOEC (10 mg/kg/day and 31 mg/kg/day) was noted when thyroid histological and hormonal changes were considered (EPA 1997). This lower NOEC would drastically alter the hazard evaluation for rodents.

Soil concentrations were consistent throughout each experiment. All control samples were less than 0.2 mg/kg. Pretest samples ranged from 92-125% of targeted values. Post-test values in the springtail and earthworm studies ranged from 78-90% of applied. Pill bug and seedling growth studies ranged from 85-109% of targeted values. Although soil concentrations were stable, bioavailability may have decreased over the length of the study, especially in earthworm tests in which the soil was highly processed and organic material was added. The tests conducted in this study were intended to provide conservative estimates of toxicity; therefore, fresh residues were used in testing. Pendimethalin contamination in the field, especially point-source contamination such as spill sites, consists mostly of aged residues. Aging of contaminated soils often reduces bioavailability, which may result in overestimation of risk (Kelsey and Alexander 1997). However, this is not always the case, in a study using soil with low organic matter, the toxicities of pyrene and phenanthrene were not reduced after aging for 120 days (Sverdrup et al. 2002). Therefore, a conservative approach is warranted.

**Conclusions**

Persistent levels of pendimethalin, even at field application levels (10 mg/kg and below), will likely impact plant communities. Invertebrate communities are more tolerant, yet earthworms may be affected by field applications, and springtail populations would likely be entirely decimated at contamination levels 10x field application. Soil concentrations at levels of potential field application would result in little risk due to trophic transfer, but soil contaminated at a concentration as low as 3x field application could be cause for concern for birds.

Site-specific factors such as size, distribution, and bioavailability of the contamination will influence the risk assessment at a given site. Overall, this hazard evaluation indicates that even low levels of pendimethalin contamination (less than 10 mg/kg) may need to be further investigated if plant productivity is of concern and moderately contaminated sites (30 mg/kg and above) cause enough concern for further investigation, regardless of intended site usage due to the potential for trophic transfer effects. Phytoremediation of moderately contaminated pendimethalin sites (30-100 mg/kg) has been suggested (Belden 2003b). During any slow remediation technique involving pendimethalin, such as phytoremediation, environmental effects should be evaluated to insure that environmental damage is not occurring during remediation.

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# CHAPTER 6. PERSISTENCE, MOBILITY, AND BIOAVAILABILITY OF PENDIMETHALIN AND TRIFLURALIN IN SOIL

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**A book chapter accepted by J.R. Coats and H. Yamamoto (eds.) *Environmental Fate and Effects of Pesticides*. American Chemical Society, Washington, D.C.**

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## Abstract

Pendimethalin and trifluralin are current-use pesticides that have been previously reported as persistent, bioaccumulative, and toxic. In the studies presented here, dissipation of aged and fresh residues of pendimethalin and trifluralin were evaluated in soil, as well as the bioavailability of residues to earthworms and the movement of pendimethalin in a soil column. In a separate study, pond water receiving runoff from a golf course was measured for the presence of pendimethalin. Dissipation measurements of pendimethalin and trifluralin in soil indicated very slow dissipation with 40–60% of the compounds extractable at 1026 days after the first measurement. In a second study, dissipation of pendimethalin was more rapid, however more than 30% was present after 310 days of soil treatment. Biovailability, as measured by earthworm biological accumulation factors, was reduced over time. Mobility of pendimethalin was very limited. Almost no downward movement was measured in the column study, and no detectable levels were found in runoff from turf grass.

## Introduction

Dinitroanaline herbicides are used for pre-emergent control of grass weeds in many crops including soybeans, cotton, and turfgrass. In plants, these herbicides bind to tubulin, thus preventing microtubule formation, disrupting mitosis, and causing ultrastructural effects (1). Throughout the last thirty years, large amounts of both of these herbicides have been applied to soil. In 1996 alone, five million kilograms of trifluralin and six million kilograms of

pendimethalin were applied to soybean fields in the United States (2). Herbicide use can cause nonpoint source contamination of surface and groundwater. Additionally, due to the large amount of herbicide usage, incidental spillage during mixing and loading can occur, resulting in heavily contaminated soil (3).

Previous studies have indicated that soil found at agrochemical dealerships in Iowa had concentrations of pendimethalin above 100 mg/kg and concentrations of trifluralin above 10 mg/kg (4), levels well above application rates. Since the degradation rate of some pesticides is concentration-specific, with higher concentrations degrading more slowly (5, 6), the environmental impact of these sites may not be well predicted by current fate studies, which are mostly conducted using field conditions.

Pendimethalin and trifluralin have low water solubility and sorb to soil at a high rate. However, some studies have indicated that pendimethalin and trifluralin may contaminate groundwater and surface water. For instance, pendimethalin has been found in groundwater near agrochemical dealerships (3) and both trifluralin and pendimethalin have been detected in surface water in studies conducted by the United States Geological Survey (7).

The presence of herbicides in surface and groundwater is widely studied because of the potential impact on human and environmental health. Although less studied, soil contamination can also impact the environment in a variety of ways. First, the contaminant can move into aqueous compartments by leaching into groundwater or entering surface water through runoff. Secondly, the chemical can cause local effects due to direct toxicity to plants or animals. Finally, the contaminant can accumulate in local fauna or flora and cause toxicity to animals at higher trophic levels.

Both of the most intensively used dinitroanaline herbicides, pendimethalin and trifluralin, have been placed on the United States Environmental Protection Agency's list of Persistent Bioaccumulative and Toxic chemicals (PBT; 8). Most of this list is composed of industrial and past-use pesticides such as chlorinated insecticides, mercury, polychlorinated biphenyls, polyaromatic hydrocarbons, and DDT. The dinitroanalines are the only compounds on the PBT list that are currently produced in the United States for release into the environment at high levels, although the environmental fate and effects of dinitroanilines have been less studied as compared to other PBTs.

In the past few years we have conducted a variety of studies to determine the fate of herbicides in soil. Each study investigated several herbicides and was designed to evaluate either best management practices for reduction of pesticide runoff or phytoremediation of herbicide residues in soil. The portions of each study regarding pendimethalin or trifluralin movement and dissipation are summarized within this manuscript to provide an overview of the environmental fate data we have collected regarding dinitroanaline herbicides. Complete studies reporting data for all pesticides involved and all remediation strategies will be published elsewhere.

## Methods

### *Microplot Study*

Soil from an agrochemical dealership site (loamy sand, 1.6% organic matter) was treated with 100 ppm atrazine, 25 ppm metolachlor, and 25 ppm trifluralin in addition to the 110 ppm of pendimethalin and 10 ppm metolachlor already present. The soil was distributed between four containers (24 x 30 cm base with 18-cm depth). Microplots were placed outside during the summer months (near a cornfield on the Iowa State University campus) and within a greenhouse during winter months (20°C, 16:8 light to dark). Soils were aged for thirty days before the first sampling. Plots were sampled by taking three soil cores at various points in time up to 1026 days. Concentration of the pesticides was measured at each time point. After 1026 days, soil from each plot was allowed to dry and then mixed thoroughly. Chemical analysis and soil earthworm bioaccumulation studies were performed to evaluate the bioavailability of the remaining dinitroanaline residues.

*Extraction and Analysis of Soils*

Soils were extracted as previously reported (9). Briefly, 20 g of soil were shaken three times with 60 ml ethyl acetate. The resulting extract was concentrated either under nitrogen flow or by a rotary evaporator. Analysis of the extracts was performed by gas chromatography and thermionic specific detection (GC-TSD) as previously described (9).

*Earthworm Bioassay*

Eight-day earthworm bioaccumulation assays were conducted as previously reported (10). Four adult worms (*Eisenia fetida,* average mass 1.5 g) were placed in 200-ml jars with 150 g of the test soil. Soil moisture was adjusted to 19% (approximately 1/3 bar) before

sealing the jar with perforated Parafilm®. After eight days of incubation at 25°C and constant low level light, earthworms were removed from the sample soil and placed in untreated soil for one day. Worms were then removed and extracted three times with 10 ml of ethyl acetate in a sample homogenizer. Measurement of trifluralin or pendimethalin was then performed using GC-TSD. Biological accumulation factors (BAFs) were calculated as concentration in the earthworm divided by the concentration recoverable by ethyl acetate extraction, and these BAFs were used as a measure of bioavailability. Control studies were performed identically using soil that had been fortified with herbicide 24 hours before the start of the test. Control studies provide a baseline for the expected BAFs for pendimethalin and trifluralin for fresh residues.

***Soil Column Study***

Eight soil columns were constructed in PVC pipe (10 cm in diameter, 23 cm long) enclosed at the bottom with aluminum screen and glass wool. The soil used was collected from a corn field near Ames, IA that had not received herbicide treatment (sandy loam, 2.7% O.M.; Field 55, ISU Ag Engineering/Agronomy Farm). The columns were packed with 7 cm of unfortified soil at the base of the column, and topped with 14 cm of soil fortified with atrazine, alachlor, metolachlor, and pendimethalin each at 25 mg/kg (36.9 mg per column). Columns were packed to a bulk density of 1.25 g/ml. Distilled water was added slowly (1 cm/hour) to each column to reach a moisture content of 1/3 bar (19.1%). Columns were maintained at greenhouse conditions (25°C, 16:8 minimum light to dark). Each column was watered with 1 cm (81 ml) of distilled water every 96 hours. This amount kept the columns moist, yet was insufficient to cause loss of water from the bottom of the column.

*Leaching of Soil Columns and Removal of Soil*

After 240 and 330 days, 7.5 cm (608 ml) very soft water (12.0 mg/L as NaHCO3) was added to each column over an 8-hour period. Leachate was collected at the bottom of the column. Leachate was stored at 4°C no longer than 48 hours before analysis by solid phase extraction. Extracts were analyzed by GC-TSD.

The columns were then left at greenhouse conditions without water for ten days after the leaching process (250 and 340 days). Soil from each column was collected from three regions: the top 6 cm, the following 6 cm (middle), and the bottom 5 cm. The soil was

sieved (2.8 mm) and then stored in glass containers at 4°C. Chemical analysis and earthworm bioassays were conducted as previously discussed.

***Golf Course Study***

Water samples were collected from Braeburn Golf Course in Wichita, KS. The course is an 18-hole, 160-acre, public golf course that contains four ponds. Pendimethalin was applied as a pre-emergent herbicide on the fairways of the course throughout the sampling period. The formulation applied was a fertilizer and pendimethalin mixture (Scott's Brand) applied at 134 kg/ha (active ingredient 1.2%, 1.6 kg/ha). Samples were collected from two ponds. Pond A receives runoff from the southern and western portions of the course, as well as flow from a pond not studied, and flow from drainage tiles. Pond A is small (800 $m^2$) and shallow (<0.3 m deep). Pond B receives flow from fairways and from an unstudied pond. It is a larger (3000 $m^2$) and deeper (average 2.5 m) pond. Water draining from Pond B leaves the golf course and enters the Arkansas River Watershed. A more thorough description of the site and sampling techniques is available elsewere (11).

The study lasted for three years, from July 1997 to October 2000. Samples were taken from the two ponds biweekly. In addition, water was collected during rain events five times in the first two years and ten times in the third year of the project. Rainfall had to exceed 1.5 cm to qualify as a rain event. During each rain event, samples were collected 2, 4, and 24 hours following the onset of rain. Samples were collected approximately 0.5 m from the shoreline as grab samples in 1 L glass bottles. Samples were transported to the laboratory and analyzed within 48 hours.

Pesticides were extracted by solid-phase extraction and analyzed by gas chromatography coupled with nitrogen-phosphorus detection as previously described (12). Samples were fortified with tributylphosphate in acetone as a surrogate. Recovery for pendimethalin using this method was 80.5 % with a standard deviation of 6.8 % (n = 36). Quantitation limits were 0.4 μg/L.

## Results

***Microplot Study***

Pendimethalin and trifluralin had very low dissipation rates. As shown in Figure 1, both compounds had very little dissipation after the first thirty days of aging. Only small losses

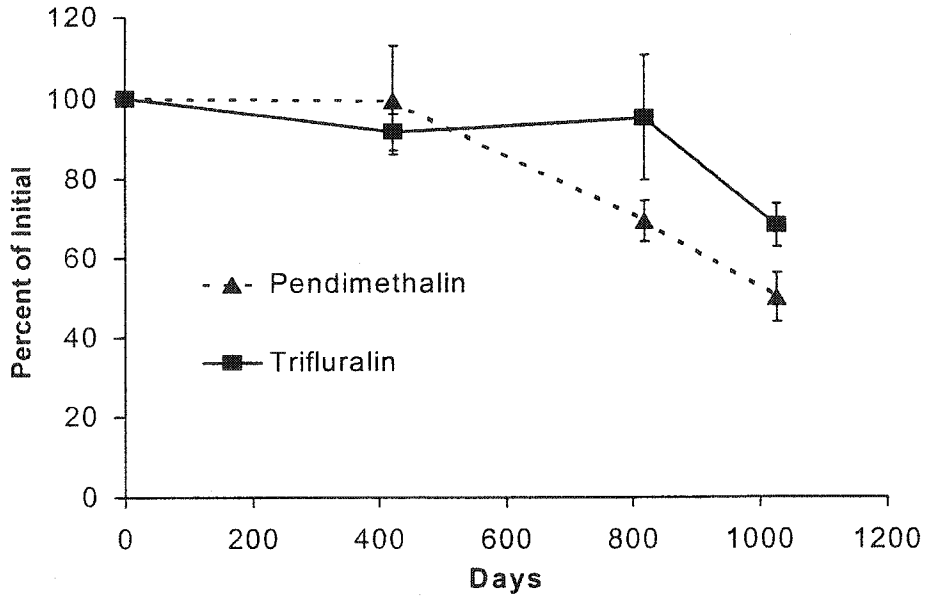


*Figure 1. Dissipation of pendimethalin and trifluralin in field microplots. Error bars illustrate one standard error.*

occurred throughout the first year, and greater than 50% of each compound was present after 1000 days. At the end of the study, a higher percentage of pendimethalin had dissipated as compared to trifluralin, even though it was present in the soil at higher concentrations and was aged for a longer period prior to the beginning of measurement collection.

Pendimethalin and trifluralin accumulated in earthworms. After eight days of exposure to soil from the microplots, BAF values were calculated as 2.9 (SE 0.2) for trifluralin and 0.78 (SE 0.08) for pendimethalin. BAF values calculated for exposure in control soil (as reported for the column study) aged for only one day were 5.7 (SE 0.5) for trifluralin and 1.9 (SE 0.2) for pendimethalin. For both pendimethalin and trifluralin, the BAFs were significantly lower in microplot soil as compared to freshly treated soil ($p < 0.01$).

***Column Study***

Dissipation of pendimethalin in the study involving soil columns also was slow. After 250 days in the soil column, 41% (SE 2) was present, and after 340 days, 31% (SE 2) was

present. As shown in Figure 2, dissipation was significantly faster in the top of the column than in the middle section ($p < 0.05$) and was greater as time increased ($p < 0.05$). In addition, pendimethalin moved very little in the soil column. Pendimethalin was not detectable in the leachate (less than 0.05% of amount applied), and less than 1.5% of the applied amount moved into the bottom unfortified soil section.

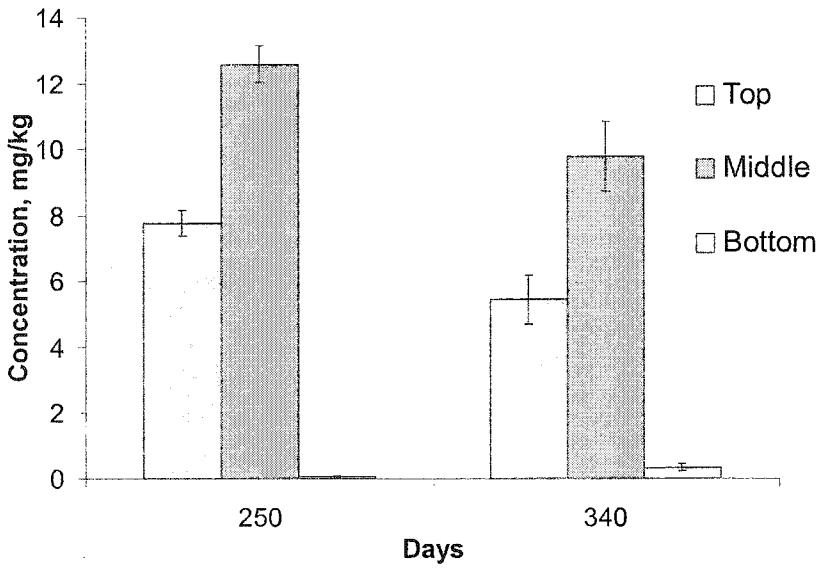


*Figure 2. Concentration of pendimethalin in soil column sections following aging and leaching. Twenty-five mg/kg of pendimethalin was applied to the top two sections of the column. Length of time in the soil and section position both significantly affected the amount of pendimethalin remaining in the soil.*

Pendimethalin also was detected in earthworms exposed to soil from the top and middle sections of the columns. As shown in Figure 3, bioavailability dropped in all sections and times as compared with the BAF values calculated for exposure in control soil (1.9, SE 0.2). BAFs were significantly lower for the top section as compared with the middle section ($p <0.05$) and for columns aged 340 days as compared with 250 days.

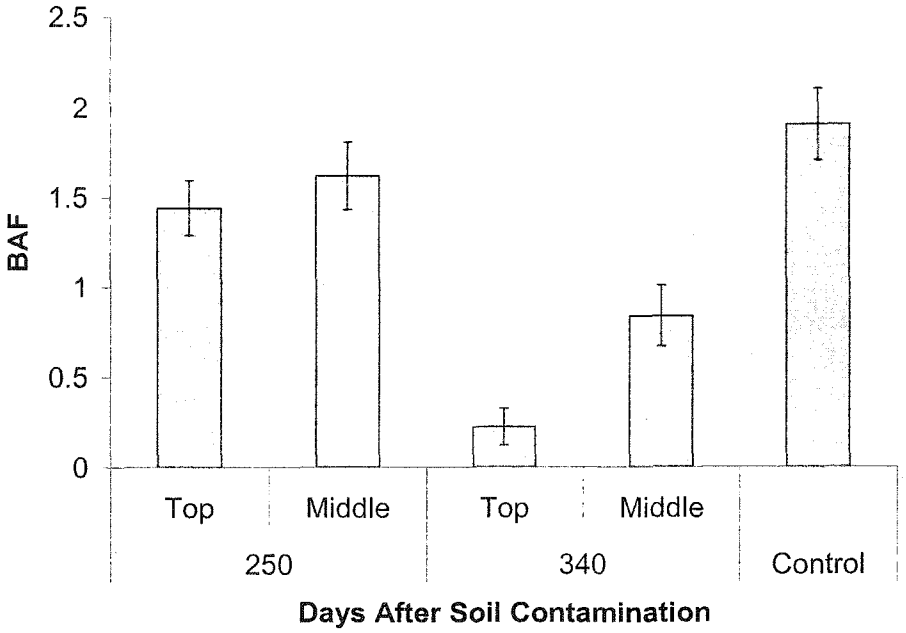


*Figure 3. Biological accumulation factors (BAFs) for pendimethalin accumulation in earthworms. Greater length of time in soil and location in the top of the soil column significantly decreased BAF values. Control value was determined with freshly fortified soil*

### *Golf Course Study*

Pendimethalin was not detected in any of the samples collected in this study. Other compounds, such as simazine, which were applied to the fairways, were detected in this study as previously reported (11). Quality control for the analytical procedures demonstrated the effectiveness of the measurement method. Matrix spike and surrogate recoveries were above 70% for all samples.

## Discussion

These results demonstrate the persistence of pendimethalin and trifluralin. At the end of the microplot study, less than 50% dissipation had occurred for both compounds, indicating that the half-life of the residues was greater than 1000 days. In the column study, the dissipation rate of pendimethalin was faster, yet after 340 days, 31% still remained. Although the number of time points was limited, the half-life of the residues could be estimated as greater than 170 days, as an average of the first two half-lives. The difference in

pendimethalin dissipation rates between the studies may be the result of several factors. First, the agronomic soil used in the column study had higher organic matter, higher clay content, and may have been more biologically active than the sandy, previously contaminated soil used in the microplot study. But, increases in organic matter content usually result in the reverse trend with greater adsorption to the soil. Second, in the column study, soil was kept at 25°C and at moisture conditions close to the gravimetric water potential of field capacity throughout the study, while in the microplot study, soil was left outdoors, subject to temperature and moisture extremes. At times, these conditions were likely to be cooler and dryer than ideal for degradation. Pendimethalin has been shown to dissipate more slowly with decreased soil moisture (14). Finally, pendimethalin within the microplots had been aged for an unknown amount of time before the start of the test, and therefore may not have been available for volatilization or biodegradation.

Previous studies have often reported shorter dissipation half-lives for pendimethalin and trifluralin. For instance, pendimethalin has been reported to have dissipation half-lives of 37 days in onion fields (13), 47 days in agronomic soil (14), and 12 days in thatch on a golf course (15). Other laboratory studies have reported pendimethalin half-lives as long as 98 days at 30°C and 407 days at 10°C (16). Trifluralin residues have been shown to persist in fields, with half-lives of 35.8 and 25.7 days (17) or longer (18). However, additional studies have indicated that after a period of initial dissipation, both compounds may have much slower dissipation rates (17, 18). Concern has been raised about the validity of using dissipation rates as a measurement of persistence. Degradation half lives for both compounds may be much longer (8). But, dissipation rates were measured in these studies and, in our opinion, are relevant to soil concentrations in the field.

The dissipation rates found in both the microplot and column studies could be slow for a variety of reasons. Both trifluralin and pendimethalin may have dissipated due to volatility and photodegradation (18, 19, 20). Rapid incorporation of the pesticides into the soil during these studies may have decreased the magnitude of these important dissipation mechanisms. The concentrations used in these studies were also higher than those used in previous studies, in order to represent point-source spillage instead of field application. Increased concentration has been shown to decrease degradation rates for some compounds (5, 6).

Pendimethalin was shown to have minimal mobility in surface runoff and leachate in this study, as demonstrated by the lack of movement in the soil column study, and the less-than-detectable levels found in the ponds in the golf course study. These results are not unexpected. In general, the dinitroanalines have low water solubility and high soil adsorption (18). Field studies of pendimethalin have indicated very little leaching through soil columns (21). This lack of movement, coupled with previous reports of higher dissipation rates of pendimethalin on golf course thatch (15), may explain why detectable water contamination was not present on the golf course. However, if contamination of the water did occur, pendimethalin is likely to partition quickly into the sediment, which was not tested.

Overall, BAF values for pendimethalin and trifluralin in these studies decreased over time as compared to BAF values for fresh residues. This result was also expected. Many persistent contaminants have been shown to have decreased availability over time, and it has been suggested that the change in bioavailability should be considered when estimating risk (22). In the column study, the BAF values for the top section were significantly lower than for the middle section ($p < 0.05$). Due to the overall method design of the experiment, potting soil rich in organic matter was added to the top of the column. This increase in organic matter may have resulted in the decrease in bioavailability.

In summary, our results indicate that pendimethalin and trifluralin may be very persistent herbicides when incorporated into soil. However, when considering the potential environmental hazards of these residues, one should consider the low mobility of the compounds within the soil and the decreased bioavailability that occurs as the residues age.

**Acknowledgements**

Partial financial support for this project was provided by the Center for Health Effects of Environmental Contaminants (CHEEC) at the University of Iowa. This chapter is publication No. J-19837 of the Iowa Agriculture and Home Economics Experiment Station, Project 3187. Financial assistance has also been provided by an EPA Section 319 Non-Point Source Pollution Control Grant C9007 405-97 through an agreement with the Kansas Department of Health and Environment. We would also like to mention John Wright golf course general manager, and superintendent.

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# CHAPTER 7. DETOXIFICATION OF PESTICIDE RESIDUES IN SOIL USING PHYTOREMEDIATION

**by J. B. Belden, B.W. Clark, T. A. Phillips,**
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**A book chapter accepted by J. Gan (ed.) *Remediation of Pesticides*. ACS, American Chemical Society, Washington, D.C.**

## Abstract

During the past few years, we have conducted a series of experiments to investigate the potential of using plants as tools for the remediation of pesticide-contaminated soil. We have demonstrated that a blend of prairie grasses increases dissipation rates of several pesticides including metolachlor, trifluralin, and pendimethalin. However, in other studies, mulberry trees were not shown to influence pesticide dissipation. Additional studies have demonstrated that metolachlor movement in the soil column may be reduced by the presence of prairie grasses, bioavailability of dinitroanaline herbicides may be reduced during phytoremediation, and soil and leachate from remediated soil may have less toxicity than expected. Current studies within our laboratory are being conducted to determine the role of prairie grass blends in the phytoremediation procedure as compared to individual species and the role of plant uptake of pesticides in the phytoremediation process.

## Introduction

Phytoremediation – the use of plants as a remediation agent for contaminated water or soil – has recently become widely investigated as a possible solution for many pollution problems. For example, plants have been used to remove heavy metals from soil, grasses have been used to remediate petroleum hydrocarbons, industrial solvents, and explosives from contaminated soil, and trees have been used to remove atrazine and industrial solvents from groundwater plumes (1). Phytoremediation is able to remediate such diverse contaminants due to the variety of mechanisms plants may use to either remove or detoxify contaminants. Heavy metals and some organic compounds may be removed by plant uptake such as the case for removal of heavy metals from soil and removal of volatile-organic

compounds from groundwater. Organic compounds may be further degraded in the plant, while heavy metal uptake requires removal of the plant from the site. Plants are also capable of increasing degradation of organic compounds in the rhizosphere (root zone of the plant). This is often due to the plant releasing exudates from their roots, resulting in increased microbial activity (1); however, a few investigators have reported direct release of degrading enzymes, capable of biotransformation of organic compounds (2).

Our current research has focused on potential for using phytoremediation for cleanup of point-source pesticide contamination. During the manufacturing of pesticides, distribution to agrochemical dealerships, mixing of formulations, and loading of pesticides into tanks for application, there is a great potential for the occurrence of pesticide spillage. In fact, one study estimated that 90% of agrochemical dealerships in Iowa have pesticide-contaminated soil and 50% of these sites will need remediation (3). High concentrations of pesticide contamination in soil can impact the environment in several ways, including leaching into groundwater, running off into nearby surface water, or directly impacting local soil organisms.

We have mostly concentrated on a set of pesticides – atrazine, metolachlor, pendimethalin, and trifluralin – that have been among the most heavily used pesticides in the corn-belt region of the United States for many years (4). Atrazine and metolachlor are moderately persistent in the environment and are relatively soluble in water. Studies have shown that they are two of the most common contaminants of ground and surface water (5). Pendimethalin and trifluralin are not very mobile in soil (6); however, they are persistent and tend to bioaccumulate (7).

In a series of studies, we have investigated the potential use of individual prairie grasses, a prairie grass mixture, and mulberry trees for the phytoremediation of these pesticides. Our initial experiments have been conducted to demonstrate that plants can survive in soil moderately contaminated with pesticides, and their presence increases the degradation rate of pesticides. Further experiments have been designed to evaluate the remediation system by examining pesticide movement in the soil column during remediation and evaluating bioavailability of the remaining residues. The latest experiments have been focused on the mechanisms involved with our phytoremediation strategy, including the impact of mixed grass species as compared to individual species and the amount of pesticide that is taken up

by the plant versus degraded in the soil. The purpose of this chapter is to review our recent and current phytoremediation investigations. Full detail of the experiments is being published elsewhere.

**Evaluation of Plants for Phytoremediation Potential**

The prairie grasses we are investigating – yellow indiangrass, switchgrass, and big bluestem – are deep-rooted perennial plants with long growing seasons, they are tolerant to moderate levels of pesticides, and are commonly available as seed. Prairie grasses have also been shown to have phytoremediation potential for other organics such as petroleum hydrocarbons (8). We have chosen to use a mixture of the three grasses in most of our studies, because mixtures are used for prairie restoration and may have a wider range of degradative capacities in their rhizosphere.

*Prairie Grasses - Microplot Study*

In a four-year study, we investigated the effect of prairie grasses on the rate of pesticide dissipation in soil obtained from a contaminated agrochemical dealership site. The soil (loamy sand, 1.6% organic matter), originally containing 110 mg/kg pendimethalin and 10 mg/kg metolachlor, was treated with 25 mg/kg atrazine, trifluralin, and metolachlor. Microplots were constructed in plastic tubs (24 x 30-cm base and 18 cm-depth), which were kept in outdoor plots in Ames, IA during the summer and in the greenhouse during winter months. After an initial aging period of 30 days, individual microplots were either planted with a mixture of the three prairie grasses, or left unvegetated (n=4). Each microplot was sampled by taking three soil cores at various points in time up to 1,026 days. After 1,026 days, soil from each plot was allowed to dry and then mixed thoroughly. Soil samples were extracted with ethyl acetate and the concentration of pesticides was determined by gas chromatography with thermionic specific detection (9).

Initial measurements taken over the first 200 days of this study indicated a trend of increased atrazine and metolachlor dissipation in the prairie grass plots. However, by the end of the study, less than 2% of the atrazine and less than 15% of initial metolachlor was recoverable. At this point, there was no significant difference between unvegetated and vegetated treatment of soil for either compound. The dinitroanaline herbicides, pendimethalin and trifluralin, were much more persistent. As shown in Figure 1, greater than

40%, and up to 70%, of the residues remained after 1,000 days. Lower amounts of dinitroaniline herbicides (pendimethalin and trifluralin) were recoverable from soil vegetated with prairie grasses ($p=0.004$). Individually statistical analysis did not show vegetation differences for pendimethalin, however the percentage of trifluralin residue remaining was significantly lower in vegetated columns (t-test, $p<0.05$).

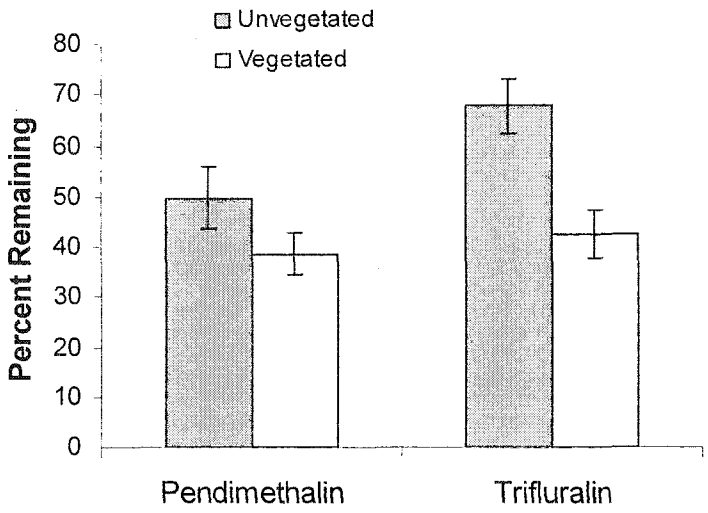


*Figure 1. Vegetation with prairie grasses significantly decreased the percentage of pendimethalin and trifluralin remaining in soil after 1,000 days of remediation ($F = 12.6$, $p = 0.004$).*

*Prairie Grasses - Column Study*

Further investigation of the prairie grass mixture for phytoremediation of pesticides was conducted using artificial soil columns. Eight soil columns were constructed in PVC (polyvinylchloride) pipe (10-cm diameter and 21-cm depth) with the bottom of the column secured with glass wool and aluminum screen. The base of the column was packed with 7 cm unfortified soil from an agronomic site that has not received pesticide application for over 15 years (sandy loam, 2.4% organic matter). An additional 14 cm of the same soil fortified with atrazine, alachlor, metolachlor, and pendimethalin at 25 mg/kg was added to the top of the column. The bulk density of the soil in the columns was 1.2 g/cm$^3$. After aging the columns for 60 days, half of the columns were planted with plugs of the prairie grass mixture previously described, while the remaining columns were left unvegetated.

The columns were placed in a greenhouse for 240 days and watered as needed to keep the columns moist, but not to cause water to come out of the bottom of the column. At the end of the study, a "storm event" was performed which entailed adding 608 ml water to each column (corresponding to 7.5 cm of rain). This amount of water resulted in leaching through the column. The leachate was extracted by solid-phase extraction. After 10 days without water, the columns were divided into three soil profile regions, and the soil was extracted by shaking with ethyl acetate. Extracts were analyzed by gas chromatography with thermionic specific detection. Full methods for the analysis techniques have been previously reported (9, 10).

Alachlor and atrazine degraded rapidly in the columns; less than 2% of the applied amount was recovered from the system and no differences were found between vegetated and unvegetated treatments. As shown in Figure 2, vegetation with prairie grasses did significantly reduce the total amount of metolachlor ($p<0.01$) and pendimethalin ($p<0.01$) recoverable from the soil column and leachate.

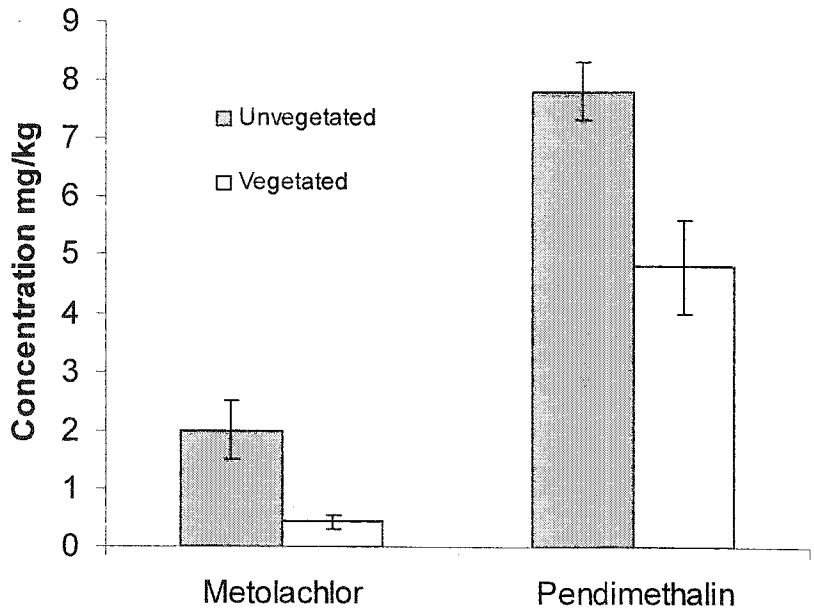


*Figure 2. After 250 days of remediation, artificial soil columns planted with prairie grasses had significantly less extractable metolachlor and pendimethalin as compared with unvegetated soil columns.*

*Mulberry Trees*

Mulberry trees (*Morus rubra*) have been suggested as potential phytoremediation tools due to root structure and composition of root exudates (11). This rapid-growth species of tree thrives in the Midwestern U.S.; therefore, we designed a study to investigate their use as a phytoremediation tool for pesticides. Soil fortified with 100 mg/kg atrazine, and 25 mg/kg trifluralin and metolachlor, was packed inside PVC pipe 15 cm in diameter and 30 cm long. Columns were allowed to age for 60 days to better reflect the type of pesticide residues found at agrochemical dealerships. Subsequently, ten artificial soil columns were planted with mulberry trees, and ten were left unvegetated. After 170 days, soil from 5 vegetated and 5 unvegetated columns was measured for pesticide concentration. Measurements were taken from the remaining columns at 330 days. Soil concentrations were determined using solvent extraction of the soil followed by analysis by gas chromatography and thermionic specific detection as previously described (9).

The trees grew at a slower rate than expected. Examination of the roots revealed limited growth in a twisted pattern, leading us to believe inhibition of root growth occurred, likely as a result of trifluralin. As shown in Figure 3, the presence of mulberry trees did not reduce the amount of pesticide present as compared to unvegetated controls. In fact, the mulberry containing soil columns contained significantly higher levels of metolachlor than did unvegetated columns ($p<0.01$). Several factors may influence the potential of mulberry trees for phytoremediation. First, as with all phytoremediation, damage to the plant by the contaminant may reduce the impact of the plant. Second, mulberry trees have been reported to release exudates in seasonal cycles with a greater release of phenols in the fall during senescing (11). However, because this experiment was conducted in a greenhouse, environmental factors may not have been appropriate to result in high releases of exudates. Third, the release of phenolic compounds in root exudate is likely to cause a shift in microbial populations (11). As a result, this shift may increase or decrease the degradation of contaminants on a contaminant-specific basis. Finally, soil obtained for this study had a previous history of metolachlor treatment. Therefore, if a population of microbes with metolachlor-degrading capabilities was already present, a decrease in this microbial activity in the mulberry columns may account for the results.

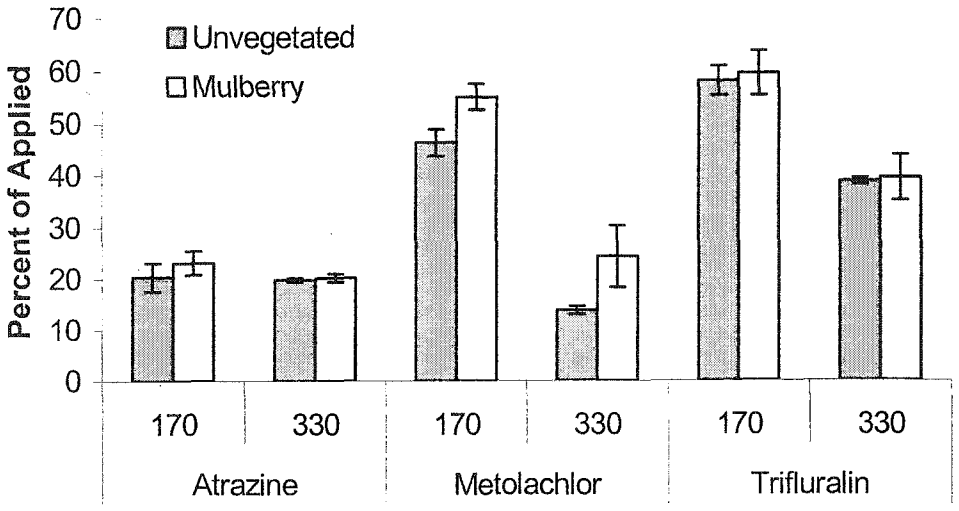


*Figure 3. Percentage of applied atrazine, metolachlor, and pendimethalin recovered from columns as a whole at day 170 and 330. Presence of mulberry trees did not affect the percentage of atrazine and trifluralin remaining; however, mulberry did significantly increase the percentage of remaining metolachlor ($p<0.01$).*

*Evaluation of Phytoremediation Success Using Alternative Endpoints*

Phytoremediation, as with all bioremediation, may take an extended period of time before remediation is successful. During this time, movement of the pesticide or metabolites generated in the process into biota, surface water, or ground water may cause undesirable environmental effects. In order to evaluate our phytoremediation method thoroughly, we have evaluated many of our phytoremediation studies using alternative endpoints, in addition to the traditional approach of chemically measuring the concentration of the contaminants of interest.

*Pesticide Movement within the Soil Column During Phytoremediation*

In the study previously described as the "Column Study", concentrations of pesticide were measured throughout the column and in leachate recovered from the bottom of the column after a "storm" event. Figure 4 illustrates the percentage of total recoverable metolachlor obtained from the leachate. Interestingly, the vegetated columns not only reduced the total amount of metolachlor present as previously noted (see Figure 2), but also decreased downward leaching of metolachlor. After 160 days of remediation, vegetation

resulted in a two-fold decrease in the total amount of metolachlor recovered from the system, while a five-fold decrease was noted for the amount of metolachlor that was recovered from leachate. After 250 days, the presence of vegetation resulted in a four-fold decrease recorded for total metolachlor, while a 20-fold decrease was noted for leachate.

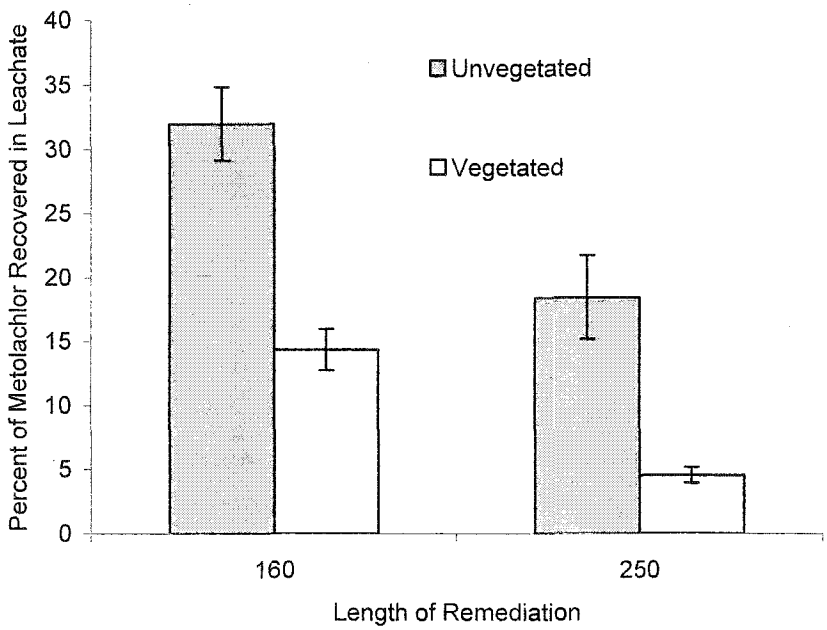


*Figure 4. Percentage of total metolachlor recovered from the system, that was recovered in leachate. Significantly less metolachlor was recovered in leachate from vegetated columns as compared with unvegetated columns ($p<0.01$).*

**Bioavailability of Pesticide Residues**

As compounds age in soil, they often have reduced bioavailability, potentially resulting in decreased environmental risk (12); however, the rate of bioremediation may also decrease. During phytoremediation, plant-induced changes in the soil environment may potentially change bioavailability as well; therefore it is important to monitor bioavailability while evaluating phytoremediation techniques. We have used two main assays for determining bioavailability in remediating soil. The first assay, an 8-day earthworm bioavailability test, was conducted as previously described (12). Earthworms *(Eisenia fetida)* were exposed to the contaminated soil, followed by analysis of the worms and test soil for pendimethalin and metolachlor. The ratio of the concentration in the worm compared to the concentration in the

soil was determined (biological accumulation factor; BAF). The bioassay was conducted on soil immediately after fortification of pendimethalin and with soil obtained during the phytoremediation experiment previously described as the Column Study. The ratio of the BAF determined for the remediated soil was divided by the BAF determined for fresh residues to obtain percent bioavailability for earthworms.

The second bioassay used to evaluate bioavailability was lettuce seedling growth. Percentage inhibition was determined seven days after adding seeds to soil. Multiple concentrations of pendimethalin and metolachlor were evaluated as fresh residues to obtain dose-response relationships. Seedling growth was then measured in soil obtained from the previously described Column Study. Comparison of pesticide concentrations in the Column Study soil to the dose-response data obtained for each pesticide suggested that pendimethalin was the major toxicant in the system to lettuce. Therefore, the seedling inhibition rates determined for Column Study soil were used to calculated effective soil concentrations using the dose-response curve for pendimethalin. The effective concentration was then divided by the measured soil concentration of pendimethalin to obtain the percentage bioavailability for lettuce.

As shown in Figure 5, the bioavailability of pendimethalin, as measured by the earthworm assay and the lettuce assay, was reduced by the presence of vegetation and by section after 160 days. The section effect is likely due to the addition of organic matter (in the form of potting soil) into the top section during the addition of plants (and potting soils plugs into controls). Vegetation also adds organic matter to soil and may increase microbial activity and the turn-over rate of organic matter. These processes could be responsible for the vegetation related decrease in bioavailability.

The decrease in bioavailability was of greater magnitude for lettuce as compared to earthworm uptake. The difference is likely due to the uptake mechanism differences between the species (plant and animal). Lettuce uptake is primarily through partitioning of the toxicant from soil, into soil water, and then into the plant root. Since earthworms ingest soil, enzymes and other gut factors may aid in the uptake of the pesticide. Chemical differences between lettuce and earthworm cuticles may also be very important. When calculating bioavailability, it is important to acknowledge that large differences may exist between types of biota, and between biological and chemical endpoints.

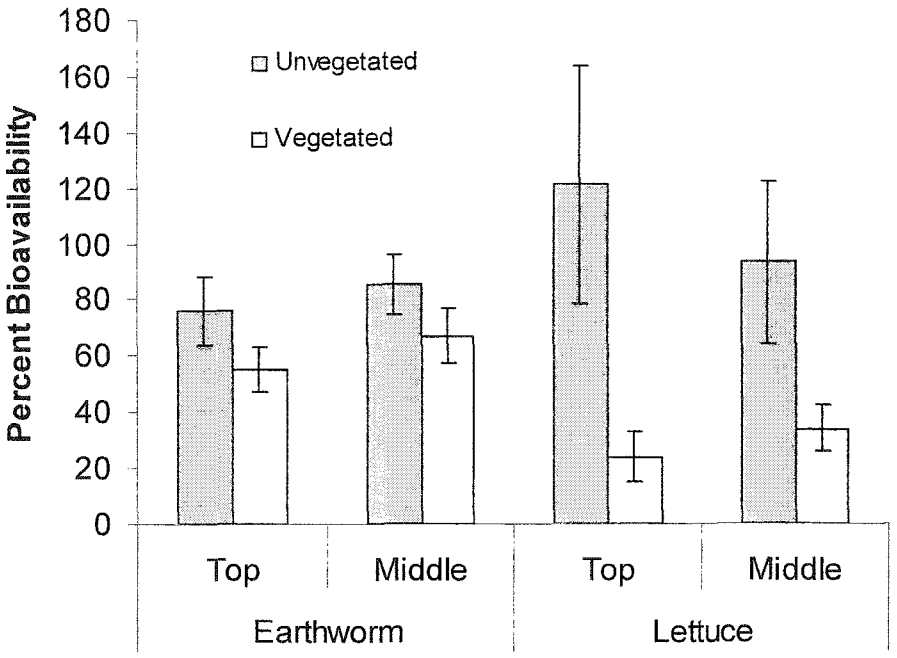


*Figure 5. Bioavailability of pendimethalin as measured by earthworm uptake and lettuce seedling growth was reduced by the presence of vegetation ($p<0.05$, $p<0.01$, respectively) in the top and middle sections of soil columns.*

**Current Research in Understanding and Improving Phytoremediation Techniques**

In order to improve and fully utilize phytoremediation for cleaning up pesticide-contaminated soil, we need more basic knowledge about the process. We are actively conducting research in two areas that should improve our understanding of the role of plants within the system. In the first area, we are evaluating the fate of radiolabeled pesticides within prairie grass-soil systems. Plants may increase dissipation rate through uptake of the pesticide or through increased degradation in the rhizosphere. Understanding the role of plants is crucial in efforts to improve phytoremediation technologies. Currently, separate studies investigating the fate of atrazine, metolachlor, and pendimethalin are being conducted.

The second area of study involves evaluating the role of species type and species mixture on phytoremediation capability. In one experiment, we investigated the effect of prairie

grasses, individually and as a mixture, on the dissipation rate of pendimethalin in a sandy loam soil (2.7% organic matter) collected from a cornfield near Ames, IA that has a history of no pesticide treatment. The soil was fortified with 25 mg/kg pendimethalin and aged for 30 days. After aging, 500 g of treated soil was added to each of 20 cones (6.5-cm diameter and 25.4-cm depth), and plugs were added: potting soil only (control), big bluestem, yellow indiangrass, switchgrass, or a mixture of all three prairie grasses (4 reps/treatment). After 180 days of remediation, soil from each cone was mixed thoroughly, extracted with ethyl acetate, and analyzed by gas chromatography (9).

After 180 days, significantly lower amounts of pendimethalin were recovered in soil vegetated with switchgrass, big bluestem, and a mixture of all three prairie grasses compared to unvegetated soil ($p<0.05$; Figure 6). These results indicate that switchgrass and big bluestem are more effective at increasing dissipation rates of pendimethalin as compared to yellow indiangrass. The mixture of prairie grasses had a similar effect on dissipation of pendimethalin as big bluestem and switchgrass. Switchgrass had the least biomass at the end of the study, indicating that the results are not strictly tied to growth and productivity of the grasses.

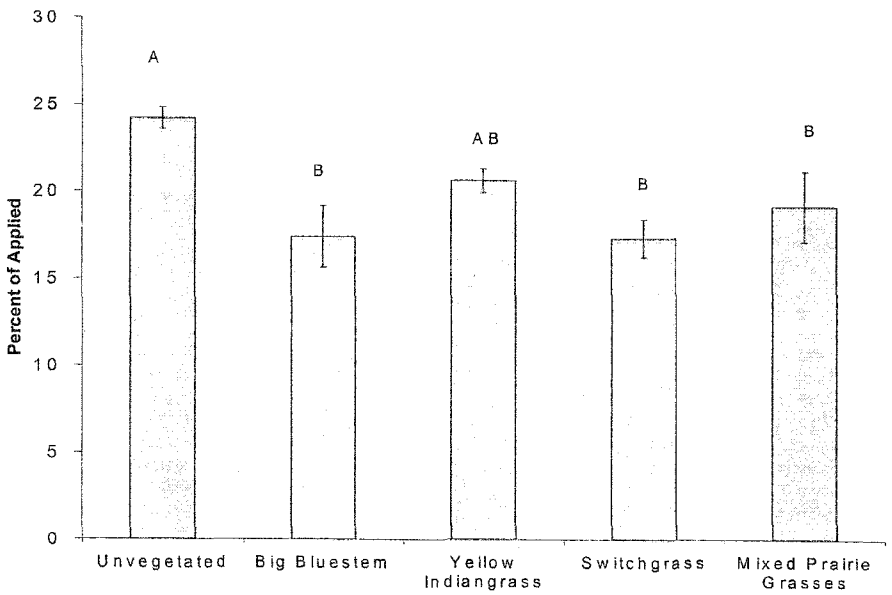


*Figure 6. Big bluestem, switchgrass, and mixed prairie grasses all significantly reduced the amount of recovered pendimethalin after 180 days of remediation ($p<0.05$). Treatments with the same letter are not significantly different.*

## Conclusions

We have found substantial evidence that the presence of vegetation can increase dissipation rates of pesticide residues in soil. Further evidence implies that vegetation may stabilize the pesticide residues, decreasing the potential for leaching and uptake into biota; thus, phytoremediation may prove to be a valuable tool in clean up of moderately contaminated sites. However, several considerations should be made regarding this technology:

1]. Plant selection is crucial. In the studies presented here, some grass species increased dissipation, one grass species had little observable effect, and mulberry trees may have inhibited metolachlor dissipation. Additionally, as seen in the mulberry experiment, pesticide damage to the plant may reduce plant growth and therefore, the impact of the plant on the environment.

2]. The process is slow. Our results have demonstrated that plants may help stabilize the system, however, if severe environmental impact is imminent, other remediation may be necessary.

3] Changes in bioavailability may hinder the complete clean-up of the site. Potential exists for aged residues, which are not available to degrading organisms to remain on the site. If strict concentration-based action limits exist for the site, the remediation technique may not be successful. However, if the compounds are not available for degradation, it is likely that the compound has limited availability to sensitive organisms or potential to leach into groundwater.

Further research studying the mechanisms and factors influencing phytoremediation may help us to improve the technology by increasing the rate of pesticide degradation and increasing our capabilities in stabilizing remaining residues.

## Acknowledgements

Partial financial support for this project was provided by the Center for Health Effects of Environmental Contaminants (CHEEC) at the University of Iowa. This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 3187, was supported by Hatch Act and State of Iowa funds.

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# CHAPTER 8. GENERAL CONCLUSIONS

Throughout this dissertation, evidence was presented that prairie grasses can increase the dissipation rate of herbicides, thus serving as *phytoremediation* agents. In Chapter 2, 78% less metolachlor and 39% less pendimethalin remained in vegetated treatments as compared to unvegetated treatments. In Chapter 3, the presence of nearly all grasses tested, but specifically the prairie grasses, resulted in greater degradation of atrazine and metolachlor in soil as compared to unvegetated soil. In Chapter 4, it was also demonstrated that pendimethalin dissipation was greater in vegetated soil and that uptake into the plant and increased degradation rates in the soil could each be partially responsible. In Chapter 6, studies are reviewed confirming that prairie grasses increase the dissipation rate of pendimethalin, metolachlor, and trifluralin. This confirms early work that the rhizosphere of certain plants may increase pesticide degradation [1], and that prairie grasses may be useful as a phytoremediation tool [2].

Prairie grasses were also shown to decrease movement of pesticides both through the soil column and into biota, thus serving as *phytostabilization* agent. In Chapter 2, nearly 20% of the metolachlor in unvegetated columns leached out of the bottom of the column after application of an artificial "rain event", while only 5% was leached out of vegetated columns. In Chapter 3, it was shown that even though vegetated columns allowed infiltration of artificial surface runoff at a much faster rate, the total amount of herbicide moving through the column was held constant, and the amount leaching through after initial applications of herbicide was reduced. Additionally, in Chapter 2, the presence of vegetation decreased the bioavailability of pendimethalin as measured by earthworm uptake and toxicity to lettuce seedlings.

Throughout the initial phytoremediation studies, it was apparent that aged residues of pendimethalin are very persistent and are likely to still be present at some level following bioremediation (Chapter 2 and Chapter 5). Therefore, a hazard evaluation was performed to determine tolerable soil concentrations of pendimethalin that could remain without risk to the biota in the environment. Even low levels of pendimethalin, field application levels (10 mg/kg or less), were shown to have toxic effects on plants and earthworms. Concentrations as low as 30 mg/kg were shown to have potentially toxic effects through trophic transfer

from soil to earthworms to birds. Thus even low levels of pendimethalin may be unacceptable, or would require site-specific risk assessment to demonstrate that unacceptable environmental harm is not occurring.

*Future Directions*

Future directions of the phytoremediation research (inclusive of traditionally termed phytoremediation and filter strip technologies) need to focus on two fronts – long-term field trials with aged residues and a better understanding of the mechanisms of phytoremediation. Although results obtained thus far indicate that phytoremediation, using prairie grasses, maybe a useful mitigation strategy, the current studies suffer from being too short term. Prairie grasses take years to fully develop. As they develop they may exert an effect deeper in the soil as root structure increases, and develop more biomass per amount of soil. Both of these factors may increase plant uptake, increase rhizosphere degradation (as a more prolific rhizosphere is present), and may stabilize the contaminant as to prevent leaching and decrease bioavailability. However, a potential pitfall for this strategy is the decrease in bioavailability. If pesticide residues become too unavailable, they may accumulate due to a lack of availability to the root or associated microbes. In addition, long-term field studies will test other factors that may be present in the field such as patchy distribution of contamination. Plants may tend to grow better in less contaminated areas resulting in better plant survival, but potentially decreasing the plant effect on contaminant degradation.

As discussed and demonstrated throughout this dissertation, vegetation can influence the fate of pesticides in soil. Chapter 4 describes some of these changes for pendimethalin. Of most interest, pendimethalin, and potentially pendimethalin metabolites, entered the plant through uptake, likely through the roots. In addition an increase in soil metabolites probably indicates that increased degradation is occurring in the soil. However, this is the extent of our current knowledge regarding how pesticide dissipation could be increasing.

Several previous studies have investigated a variety of mechanisms that could be useful in phytoremediation. Poplar trees have been shown to increase dissipation of atrazine through uptake into the plant (3); likewise, squash may accumulate DDE (4). Many pesticides have been shown to degrade more quickly in the rhizosphere of plants than in non-rhizosphere soil (1, 5). Several mechanisms may account for this degradation. For example,

exudates from corn increase mineralization of pyrene, a polycyclic aromatic hydrocarbon, likely because of stimulation of soil microbes (6). In other studies, data suggest that enzymes released from Dahurian wild rye roots (as an exudate) degrades 2-chlorobenzoic acid (7) and oxireductase enzymes are released from members of several families, including Gramineae, at high enough levels to take part in oxidative degradation of soil constituents (8). Exudate release may increase the bioavailability of organic contaminants making them more available for plant uptake and microbial degradation (4). In addition, some plants may select for specific microbes as root endophytes, the endophytes may have the ability to protect the plant by degrading organics (9).

By understanding the mechanisms involved in phytoremediation, improvement of the process may be possible. Additionally, if the processes are fully understood, it may be possible to create conditions in the soil that will enhance degradation of pesticides without needing plants. This process would circumvent the problems associated with plant toxicity.

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